FFF 2007

13th International Symposium on Field-and Flow-based Separation

June 27 – 30, 2007

Salt Lake City, Utah, USA

State of Utah Center of Excellence for Biomedical Microfluidics,
University of Utah
FFF 2007

13th International Symposium on Field-and Flow-based Separation

Organized By:

State of Utah Center of Excellence for Biomedical Microfluidics,
University of Utah, Salt Lake City, Utah

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Program

Wednesday, June 27

5:00 PM  Registration Begins

6:00 PM  Welcome Reception at Officers Club

9:00 PM  

Thursday, June 28

8:50 AM  Welcoming Remarks  

Bruce Gale

9:00 AM  L1  Invited Talk: Challenges in Understanding Protein Interactions  

David Myszka

9:40 AM  L2  A Sedimentation FFF study to Monitor Bio-Interactions on Surface Modified PS Micro-Spheres  

Catia Contado, Letizia Bregola, Stefano Sforza, Valeria Cavatorta, Rosangela Marchelli, Francesco Dondi

10:05 AM  Coffee-Break

Chair: Michel Martin  

PARTICLES

10:20 AM  L3  Thermal Field Flow Fractionation of Charged Particles in Aqueous Media  

Luisa Pasti and Francesco Dondi

10:45 AM  L4  Well Characterized, Multifunctional Nanoparticles – Essential Components In Biosensor Development  

Karin D. Caldwell and Karin Fromell

11:10 AM  L5  Retention Behavior of Surface-Charged Polystyrene and Hollow Polypyrrole Particles in Sedimentation Field-Flow Fractionation  

Sun Tae Kim, Seong-Ho Choi, Jeong-Ah Yoon, Seungho Lee

11:35 AM  Chair: Steve Williams  

POSTER INTRODUCTIONS

12:00 PM  Lunch

1:00 PM  Poster Session I

2:30 PM  Sponsor  Postnova Analytics

Introduction: Bruce Gale
Chair: Kim Williams

3: 00 PM L6  Capturing Images of Erythrocytes in High-Speed Poiseuille Flow in a Microchannel  
Yangsheng Chen, and M. Keith Sharp

Enrica Alasonati and Vera I. Slaveykova

3: 50 PM  Coffee-Break

Chair: Karin Caldwell

4: 05 PM L8  Flow Field Flow Fractionation and Size Exclusion Chromatography coupled with MALLS/DRI to determine the molar mass and coil size distribution of water soluble polymers  
S. Scholz, H. Storz, D. Lohmann, W.-M. Kulicke

4: 30 PM L9  Effect of High Pressure Homogenization on Macromolecular Size and Conformation  
Lars Nilsson, Carola Rojas, Karl-Gustav Wahlund and Björn Bergenståhl

4: 55 PM L10  Thermal Field-Flow Fractionation of Acrylic Copolymers  
J. Ray Runyon, S. K. R. Williams

5: 20 PM L11  Characterization of Ultra High Molar Mass Poly(acrylamide) Flocculants using AFFFF-MALS-RI  
Mats Leeman, Mohammad T. Islam and William G. Haseltine

Friday, June 29

8: 50 AM  Welcoming Remarks  
Marcia Hensen

9: 00 AM L12  Invited Talk: Characterizing Protein Aggregation and Particle Formation using Field-Flow Fractionation  
Shawn Cao

9: 40 AM L13  Flow Field-Flow Fractionation as a Potential Utility to Proteomics  
Myeong Hee Moon, Kihoon Kim, and Dukjin Kang

10: 05 AM  Coffee-Break
10: 20 AM L14 Hollow-Fiber Flow Field-Flow Fractionation for the Analysis of Blood Lipoproteins
Rambaldi D.C., Reschiglian P., Zattoni A., Casolari S., Roessner D., Johann C., Moon M.H, Min B.-R.

10: 45 AM L15 Instrumentation Development in Sedimentation Field Flow Fractionation, from Cell Sorting to Environmental Colloids. from Concepts to Applications and Patents
Philippe Cardot, Serge Battu, James Kassab, Gaelle Begaud

11: 10 AM L16 Hydrodynamic- Acoustic Cell Sorter - HACS
Claire Ratier, Mauricio Hoyos and Pascal Kurowski

11: 35 AM Chair: Francesco Dondi

12: 00 PM Lunch

1: 00 PM Poster Session II

2: 30 PM Sponsor Wyatt Technology
Introduction: Bruce Gale

3: 00 PM L17 Iso-Dielectric Separation for Continuous-Flow Cell Screening
Michael D. Vahey and Joel Voldman

3: 25 PM L18 Study of the Phenotypic Relationship in the IMR-32 Human Neuroblastoma Cell Line by Sedimentation Field Flow Fractionation
G. Bégaud-Grimaud, S. Battu, P. Lazcoz, J.S. Castresana, M.O. Jauberteau, P. J. P. Cardot

3: 50 PM Coffee-Break

4: 05 PM L19 Clinical Dielectrophoretic Field Flow Fractionation of Mammalian Cell Mixtures
Peter Gascoyne, Jamileh Noshari, Tom Anderson and Frederick Becker

4: 30 PM L20 Hollow Fiber Flow Field-Flow Fractionation for sorting of human progenitor cells
Leonardo Cinque, P. Stephen Williams and Maciej Zborowski
Laser-Photophoretic Migration and Separation of Human Blood Cells
Hideaki Monjushiro, Yuko Tanahashi, and Hitoshi Watarai

Reception

Symposium Banquet

Saturday, June 30

Chair: Ron Beckett

Natural Colloids and Metals: Fractionation and Speciation based on Asymmetrical Flow Field Flow Fractionation
Stéphane Dubascoux, Gaëtane Lespes, Isabelle Le Hecho, Martine Potin Gautier

New Tools for Optimization of Channel Geometry and Method Development in Asymmetrical Flow-Field Flow Fractionation (AF4)
Johann C., Roessner D, Kaltenborn A, Schuch H, Schumacher W

Macrotransport Analysis of Thermal Field-Flowfractionation
Young Seok Song, and Howard Brenner

Coffee-Break

Chair: Maciej Zborowski

Anders Danielsson, Johan Revstedt, Mats Leeman, Gustaf Modig, and Karl-Gustav Wahlund

The Steric Correction on the Potential Barrier Field Flow Chromatography
Lambros Farmakis, Athanasia Koliadima, George Karaiskakis and Stella Kenda

Spectroscopic Approach to the FFF Steric Inversion Problem
S. Kim R. Williams, Ilyong Park, Edward E. Remsen

Chair: Maciej Zborowski

Lunch
1: 00 PM  
*Poster Session III*

*Chair: Martin Schimpf*  
*INSTRUMENTATION*

2: 30 PM  
L28  
**Performances of Differential Field-Flow Fractionation as an Analytical Technique: Investigation on Precision in Differential Sedimentation FFF**  
Michel Martin, Letizia Bregola, Catia Contado, Luisa Pasti, and Francesco Dondi

3: 00 PM  
L29  
**High-Temperature Flow Field-Flow Fractionation – A new FFF Technique for Polymer Characterization**  
Edwin Mes, Hans de Jonge, Soheyl Tadjiki, Thorsten Klein

3: 25 PM  
L30  
**The Development of a Magnetophoresis Instrument for the Production of Biological Labels**  
Henrik Sandin, Chris Carr, Branda L. Hutchinson, Michael D. Ward, Christina J. Hanson, John C. Martin, Robert H. Kraus, Jr, and Michelle A. Espy

3: 50 PM  
**Coffee-Break**

*Chair: George Karaskaikas*  
*INSTRUMENTATION*

4: 05 PM  
L31  
**Quadrupole Magnetic Field-Flow Fractionation: a Novel Technique for the Characterization of Magnetic Nanoparticles**  
Francesca Carpino, Maciej Zborowski, and P. Stephen Williams

4: 30 PM  
L32  
**Separation of Micron-Size Species by using the Step-SPLITT Fractionation Channel**  
Mauricio Hoyos, Natacha Callens, Pascal Kurowski, Claire Ratiera Frank Dubois, Manuel Camargo, Andrea Niño, Marcela Camacho

4: 55 PM  
L33  
**Combining Normal and Cyclical Electrical Field Flow Fractionation**  
Srinivas Merugu, and Bruce K. Gale

5: 20 PM  
L34  
**Optimization of Particle Separation In Different Elution Modes in Field-Flow Fractionation**  
Josef Chmelík
Poster Session I-Polymers and Particles

Thursday, June 28, 1.00 PM-2.30 PM

P1 Size Determination of Nano-Sphere by Field-Flow Fractionation and Pulsed Field Gradient Nuclear Magnetic Resonance
Haruhisa Kato, Kayori Shimada, Takeshi Saito, Shigetomo Matsuyama, Mie Suzuki, and Shinichi Kinugasa

P2 Fractionation and Characterization of Gold Nanoparticles in Aqueous Solution: Asymmetric-Flow Field-Flow Fractionation with MALS, DLS and UV-Vis Detection
Tae Joon Cho and Vincent A. Hackley

P3 Physicochemical Study of the Differently Degraded Sodium Hyaluronate (NaHA) by Flow Field-Flow Fractionation and On-Line Multiangle Light Scattering
Da Young Shin, Myeong Hee Moon

P4 Application of Gravitational Field Flow Fractionation and VIEEWTM Apparatus to Characterization of Irregular Shape Particles
Sousan Rasouli, Shohre Rouhani, Sara Shahabipoor and Philippe Blanchart

P5 Blood Plasma Volume Expanders
C. Lohmann, D. Lohmann, W.-M. Kulicke, T. Liebert, Th. Heinze

P6 Separation and Quantitation of Silver Nanoparticles using Sedimentation Field Flow Fractionation
Sun Tae Kim, Dong Young Kang, Won-Suk Kim, Jong Taik Lee, Hye Sung Cho, Sang Ho Kim, Seungho Lee

P7 An Approach to Characterize Industrially Important Polyacrylate Mixtures by Thermal Field-Flow Fractionation
C. A. Lohmann, J. R. Runyon, S. K. Ratanathanawongs Williams

P8 Sedimentation Field-Flow Fractionation of Polystyrene Beads Coated with IgA: Carrier Composition Effects on Complex Characterization
Letizia Bregola, Catia Contado, Francesco Dondi.

P9 Size Characterization of Perfluorocarbon Emulsion-Blood Mixture using Sedimentation Field-Flow Fractionation
Soheyl Tadjiki, Thorsten Klein and William McGhee

P10 Fractionation of Industrial Starch Polysaccharides by Field-Flow Fractionation
Justin R. Engle, S. Kim R. Williams
P11 Analysis of Airborne Particles Collected by Various Sampling Methods by Field-Flow Fractionation, PCS, AAS and ICP-MS
Chul Hun Eum, Sang Yeon Kim, Kun Han Kim, Sun Tae Kim, Dong Young Kang, Seungho Lee

P12 Characterization of Fly Ash using Field-Flow Fractionation, Dynamic and Static Light Scattering
Dong Young Kang, Chul Hun Eum, Sun Tae Kim, Seungho Lee

P13 Advances in Programmed Field Decay Thermal Field Flow Fractionation of Polymers: Direct and inverse calibration methods
Francesco Dondi and Luisa Pasti.

P14 Effects of Surface Modification on the Retention of Gold Nanoparticles in Asymmetric Flow Field-Flow Fractionation
Jiwen Zheng, Jeffery D. Clogston, Scott E. McNeil, Anil K. Patri

**Poster Session II-Cells and Proteins**

**Friday, June 29, 1.00 PM-2.30 PM**

P15 Study of Calsequestrin Aggregation by Flow Field-flow Fractionation with Light Scattering Detection
Randy Rostock, Lou Bonfrisco, Susan E. Shadle, and Martin E. Schimpf

P16 Characterization of Lipoproteins by Flow Field-Flow Fractionation
Brechtje K. Hilbers, Wim Th. Kok, and Christoph Johann

P17 Mitochondrial Proteome Analysis by Frit Inlet Asymmetrical Flow Field Flow Fractionation and Nanoflow LC-ESI-MS-MS
Dukjin Kang, Myeong Hee Moon

P18 A New Approach for High Speed Non-Gel Based Two Dimensional Proteome Fractionation: Development of Isoelectric Focusing-Multichannel Asymmetrical Flow Field-Flow Fractionation
Ki Hun Kim, Myeong Hee Moon

P19 Multidimensional Proteome Analysis of C.glutamicum Lysate Using HF-FIFFF and 2D-Nanoflow LC-ESI-MS-MS
Ki Hun Kim, Eun Jeong Ahn, Myeong Hee Moon

P20 Hyperlayer Sedimentation-FFF Preparation of Mouse Olfactory Basal cells. A Tool for Assesement of Renewal Properties of SCO spondin
Fabrice Laloué, Annie Meniel, Barbara Bessette, Gaëlle Bégaud-Grimaud, Linda Domballe, Marie-Odile Jauberteau, Philipe J.P. Cardot and Serge Battu
P21 Relationship Between Durum Wheat Dough Strength Properties and Protein Size Distribution as Determined by Flow Field-Flow Fractionation
S.G. Stevenson and N.M. Edwards

P22 Affinity-based Protein Pre-fractionation by Flow-Field Flow Fractionation
Jishan Li, Georgia Drakakaki, Yong-Jik Lee, Natasha Raikhel, Zhenbiao Yang, Wenwan Zhong

P23 Study of the Growth Rate of Saccharomyces Cerevisiae Strains using Wheat Starch Granules as Support for Yeast Immobilization Monitoring by Sedimentation/Steric Field-Flow Fractionation
Athanasia Koliadima and George Karaiskakis

P24 Enrichment of Adipose-Derived Stem Cells using Dielectrophoretic Field-Flow Fractionation
Jody Vykoukal, Daynene Vykoukal, Susanne Freyberg, Eckhard Alt, and Peter Gascoyne

P25 Bovine Serum Albumin Aggregation Studied by Asymmetrical Flow Field Flow Fraction Connected to UV, Dual Angle Light Scattering, RI and Viscosity Detectors
Gebrenegus Yohannes, Matti Elomaa, Susanne Wiedmer, Matti Jussila, Marja-Liisa Riekkoala

P26 Wall-antibody Immobilization to Hybridize Gravitational Field-Flow Fractionation for Antigen-Specific Particle Sorting
Barbara Roda, Diana Cristina Rambaldi, Sonia Casolari, Andrea Zattoni, Pierluigi Reschiglian, Mara Mirasoli, Aldo Roda

P27 Effect of Temperature on Separation and Characterization of Monoclonal Antibody using Asymmetrical Flow Field-Flow Fractionation
Soheyl Tadjiki, Evelin Moldenhauer, Shiang Gwee and Jun Liu

P28 A MEMS-based Magnetic Cell Fractionation and Detection Device: Design, Fabrication and Testing
Lee R. Moore, Pulak Nath, P. Stephen Williams, Maciej Zborowski, Shuvo Roy, and Aaron Fleischman

Poster Session III-Instrumentation and Theory

Saturday, June 30, 1.00 PM-2.30 PM

P29 Characterization of Functionalized Styrene-Butadiene Rubber by Organic Solvent Flow Field-Flow Fractionation/Light Scattering
Da Young Shin, Dae Young Bang, Hanna Kim, Myeong Hee Moon
P30  Laboratory Designed Development of Hollow Fiber Field Flow Fractionation (HFFFF) for Macromolecules Analyses, the Example of High and Polydispersed Molecular Mass: Blue Dextran
Qin Quan, Serge Battu, Philippe CARDOT

P31  Numerical Simulations of Transport Processes in Electrical Field-Flow Fractionation System
Himanshu J. Sant, Srinivas Merugu, and Bruce K. Gale

P32  Characterization of Water-Soluble Fullerene C60 Nanoparticles Using Asymmetrical Flow Field-Flow Fractionation and Atomic Force Microscopy
Soheyl Tadjiki, Shoeleh Assemi, Bogdan C. Donose, Anh Nguyen and Jan D. Miller

P33  Effect of Channel Angle on Retention of Polystyrene Micro Beads in Gravitational Field-Flow Fractionation (GrFFF)
Mi Ri Park, Sung Kwang Cho, Dong Young Kang, Sachin V. Nehete, Seungho Lee

P34  Theory for Nanoparticle Retention Time in Quadrupole Magnetic Field-Flow Fractionation
P. Stephen Williams, Francesca Carpino, and Maciej Zborowski

P35  Evaluating Flow-through Photon Correlation Spectroscopy for the Measurement of Diffusion Coefficients for Polystyrene and Proteins
J. Ray Runyon, S. Kim R. Williams

P36  Tandem Hollow-Fiber Flow Field-Flow Fractionation
Andrea Zattoni, Diana Cristina Rambaldi, Sonia Casolari, Barbara Roda, Pierluigi Reschiglian

P37  Exploration of Shear-Induced Diffusion as a Mechanism for Non Specific Crossover in SPLITT Fractionation
P. Stephen Williams, Mauricio Hoyos, and Maciej Zborowski

P38  Study of Split-Flow Thin-Channel Fractionation Using the Discrete Element Simulation Method
Myhuong Nguyen, Martin Rhodes, Kurt Liffman, Ian McKinnon and Ron Beckett

P39  In situ Visualization of Micron-Size Particles for FFF and SPLITT Fractionation
Claire Ratier, Natacha Callens, Christophe Minetti, Pascal Kurowski, Frank Dubois, Jena-Luc Aider, Olivier Dron and Mauricio Hoyos
P40  Effects of Particle-Particle Hydrodynamic Interactions on the Mean Magnetophoretic Mobility of Particle Suspensions
Maciej Zborowski, Lee R. Moore, P. Stephen Williams, Seungjoo Haam, Jeffrey J. Chalmers

P41  Improvements in Microscale Thermal-Field Flow Fractionation Instrumentation
Himanshu J. Sant and Bruce K. Gale
Abstracts for
Oral Presentations
INVITED PRESENTATION

Challenges in Understanding Protein Interactions

David Myszka

Center for Biomolecular Interaction, University of Utah, E-mail: dmyszka@cores.utah.edu

There are numerous challenges to unraveling protein interactions which constitute the proteome. While mass spec. and microscopy are addressing concentration and location issues, biosensor technology is providing key insights into the dynamics of protein interactions. Label free sensor technology has proven itself useful for resolving a wide range of molecular interactions including small molecules, proteins, nucleic acids as well as membrane associated systems. This presentation will review some of the basic features of biosensor technology and discuss future systems and applications.
A Sedimentation FFF Study to Monitor Bio-Interactions on Surface-Modified PS Micro-Spheres

Catia Contado, Letizia Bregola, Stefano Sforza\(^1\), Valeria Cavatorta\(^1\), Rosangela Marchelli\(^1\), Francesco Dondi.

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Sedimentation Field Flow Fractionation (SdFFF) is a well established elution technique, which has been successfully applied for the separation and the size characterization of a broad range of natural and synthetic colloidal particles of environmental, industrial, pharmaceutical and biological interest.

Differential SdFFF can precisely evaluate surface concentrations of human immunoglobulin G (IgG) adsorbed to polystyrene latex. The adsorption occurs without altering the IgG reactivity, as proven by the reaction with its specific anti-human IgGs. This type of immune complex, which used for assessment of a patient’s level of immune response to a particular antigen, has been detected by SdFFF by measuring the variations in the coated PS buoyancy mass.

Among with all allergic diseases, food allergy is a growing issue, which deserves the attention of always more researchers and Institutions in developed and developing countries. Although food normally doesn't provoke a response from the human immune system, in allergic patients the hypersensitivity reactions are triggered by the IgE recognition of specific sequences (epitopes) present in the allergenic proteins.

Peach allergy is the most common form of IgE-mediated hypersensitivity to fresh fruits in the Mediterranean area, as well as in the USA. Several are the allergens from peach, but the major is an allergen belonging to the family of lipid-transfer proteins (LTP).

The aim of this study was to use the SdFFF technique as an high-resolution technique to detect the interaction between the IgE produced by the human body in consequence of an allergic food reaction due to the ingestion of peaches and specific anti human IgEs.

Preliminary tests done by using IgG, IgA and anti-IgA have proven the specificity of the surface immuno-reaction, while corollary experiments have been made to individuate good fractionation conditions.
Thermal Field Flow Fractionation of Charged Particles in Aqueous Media.

Luisa Pasti and Francesco Dondi.

Department of Chemistry, University of Ferrara, Via Luigi Borsari 46, I-44100 Ferrara, I.
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Thermal Field Flow Fractionation (ThFFF) of various types of submicronic silica particles in aqueous media is experimentally investigated under an extended range of medium ionic strengths with and without the presence of surfactant. The experiments were designed to verify the applicability to submicronic particles of the theory of charged nanoparticles thermodiffusion recently proposed by Parola and Piazza\(^1\). In particular the expression for the calibration function in terms particle radius and channel temperature is derived and experimentally verified. Moreover, retention is expected to be dependent on particle surface potential and charge, and on ionic strength. These dependences are experimentally investigated and the pertinent relationships and correlations proved. The effect of heavy metal adsorption on the silica surface was investigated and significant ThFFF retention changes measured. These effects are interpreted in light of the theory\(^1\). In particular, independent measurements of the zeta potential (\(\zeta\)-potential) proved that a decrease in the surface charge of a Silica particle is a consequence of heavy metal adsorption which is, in turn, correlated to the observed decrease in ThFFF retention. The relationships between retention parameters and carrier ionic strength, particle surface charge properties are exploited on the basis of experimental data. Such relationships can be utilized to characterize the properties of aquatic colloids properties and as a guideline for ThFFF separation optimization.

Well Characterized, Multifunctional Nanoparticles – Essential Components in Biosensor Development

Karin D. Caldwell and Karin Fromell

Department of Physical and Analytical Chemistry – Section for Surface Biotechnology
Uppsala University
Uppsala, Sweden

Biosensors are analytical tools that function as selective traps for specific analytes. In addition to the trapping, each capture needs to be quantitatively converted into a signal which can be registered to yield information regarding the number of events, in turn proportional to the analyte concentration. Molecules other than the analyte can not be allowed to generate a signal and must therefore be removed from the trap by careful washing, requiring that the trap be attached to a surface. Since the signal strength will depend on the amount of traps under observation it is important to confine as many traps as possible to the read-out area and to be able to accurately measure the surface concentration of traps in this area.

Our general approach to this problem is to mount “the traps”, typically antibodies or specifically designed affinity ligands, onto nanoparticles that also contain specific linking moieties for attachment to the read-out surface. The particles lend themselves to accurate sizing and assessment of the number of affinity traps per particle. In this, the sedimentation FFF is an invaluable tool, as it permits the operator to build up the surface layer by layer performing quantifications between each layer. In addition to providing information regarding specific binding ability on the surface, this technique permits the comparison of close-packings achievable with different ligands and an optimization of the system for sensitive detection of desired analytes.
Retention Behavior of Surface-Charged Polystyrene and Hollow Polypyrrole Particles in Sedimentation Field-Flow Fractionation

Sun Tae Kim, Seong-Ho Choi, Jeong-Ah Yoon, Seungho Lee

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Surface-charged nano- or micron-sized particles can be used as adsorbent/carryer for proteins, which can then be used for sensing of a specific protein. Separation of surface-charged particles may provide a useful tool for identification and quantification of a specific protein.

Surface-charged polystyrene particles having diameters of about 300 nm were synthesized by copolymerizing styrene with hydrophilic species such as benzoic acid, boronic acid or maleic anhydride in an aqueous medium. Hollow polypyrrole particles of similar sizes were also synthesized by coating polystyrene particles with polypyrrole, followed by removal of the polystyrene core.

The retention behaviors of the surface-charged polystyrene and hollow polypyrrole particles dispersed in aqueous media were investigated in sedimentation field-flow fractionation (SdFFF) by varying the type and the concentration of the surfactant. SdFFF was tested to see if it can differentiate the polystyrene particles of different surface charges.

The aim of this study is to understand the retention behavior of surface-charged polymeric particles in SdFFF and to find optimum SdFFF conditions for separation of various surface-charged particles.
Capturing Images of Erythrocytes in High-Speed Poiseuille Flow in a Microchannel

Yangsheng Chen\textsuperscript{a} \& M. Keith Sharp\textsuperscript{b}

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An experimental system was constructed to measure the flow-induced deformation of red blood cells in the flow between parallel plates. A channel 400 \(\mu\)m wide, 2.4 mm tall and 40 mm long was formed by micromachining grooves into two plates of PMMA and bonding them together. The flow channel was placed under a microscope and a dilute suspension of red cells was forced through the channel by a syringe pump. The cells moved at up to 4 m/s and experienced shear stress as high as 5000 dyn/cm\(^2\), which made image capture challenging. To acquire still images, a pulsed ND:Yag laser with 543 nm wavelength was used for illumination. Laser pulse duration of 3 ns limited motion blur to 12 nm. The influence of laser speckle was limited by passing the laser beam through 30 m of coiled fiber optic cable to reduce coherence. The narrow depth of focus of the 16x long working distance objective effectively isolated and located cells at the plane of focus. The numerical aperture NA = 0.4 of this lens produced an Abbe diffraction distance of 719 nm, which was adequate for resolution of red cell boundaries. A digital camera was used to capture cell images. A software controller first initiated the flow, then at regular intervals opened the camera shutter, and after a delay, triggered the laser pulse. Approximately 40 images were captured during each flow event. Image analysis software provided measurements of major and minor diameters of each deformed cell after calibration of the digital calipers with a stage micrometer. Fluid shear stresses exerted on each cell were estimated from measurements of the volume flow rate and the distance of the cell from the wall in the Poiseuille velocity profile.

These data were utilized to calibrate a new, partially mechanistic constitutive model relating fluid stresses to red cell membrane deformation and failure for predicting hemolysis in flows in cardiovascular devices. The new constitutive model was used to predict lysis of cells traveling along a large number of sample streamlines in the flow through hypodermic needles, in which the flow fields were previously calculated with computational fluid dynamics. The new hemolysis prediction model was compared to previous empirical methods and was found to be the only method to successfully mimic the trends of experimental data that showed that a prototype needle with beveled entrance caused greater hemolysis than a standard needle, while a prototype with rounded entrance caused less. Future investigations will use the image capture system to study red cell deformation in more complex flows involving a range of shear and extensional fluid stresses. This image capture system has potential usefulness for other applications involving the motion, deformation, orientation and trajectory of particles in simple and complex flows.

Enrica Alasonati and Vera I. Slaveykova

Environmental Biophysical Chemistry - ISTE - ENAC, Ecole Polytechnique Fédérale de Lausanne, Station 2, Lausanne, CH-1015, Switzerland. Emails: enrica.alasonati@epfl.ch, vera.slaveykova@epfl.ch

Microbial exopolysaccharides play an important role in environmental systems, in particular flocs and biofilms [1]. Obtained by polymerase enzymes, microbial exopolysaccharide composition, structure and molecular size are highly variable, depending on the source, the season of harvest and the location. Therefore, detailed knowledge about their molecular size characteristics is required.

Asymmetrical flow field-flow fractionation (aFlFFF) is one of the best suited tools for the separation of macromolecular samples that allows to overcome some of the restrictions that limit the use of other separation techniques such as SEC [2].

In the present work, two model exopolysaccharides were fractionated and characterized by aFlFFF coupled on-line with differential refractive index (DRI) and 7-angles laser light scattering (LS) detectors. Linear alginate, produced by algae Macrocystis pirifera and branched succinoglycan, isolated from the bacterium Rhizobium meliloti 1021 were chosen. Both exopolysaccharides are negatively charged and their reliable fractionation by aFlFFF as well as result interpretation required extensive knowledge of the factors influencing their hydrodynamic behavior in the separation channel.

The systematic variations of the cross flow rate, carrier nature and ionic strength, as well as injected mass are key parameters, which should be carefully optimized to obtain meaningful molecular size parameters. In addition, the coupling of the aFlFFF with LS detection providing independent information about the radius of gyration and molar mass distributions, confirmed the importance of careful optimization of the separation parameters to avoid the discrepancies of molecular parameters determined by aFlFFF-DRI and LS. Under the optimized separation conditions, the obtained values of the weight average and number average molar masses, radius of gyration and hydrodynamic radius for both alginate and succinoglycan were in good agreement with existing literature.

Flow Field Flow Fractionation and Size Exclusion Chromatography coupled with MALLS/DRI to determine the molar mass and coil size distribution of water soluble polymers

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The knowledge of the molar mass and coil size distribution of polymers is especially important for several technological and medical applications [1-3]. To determine the molar mass and coil size distribution it is first necessary to fractionate the polymer sample into individual molar masses. Size Exclusion Chromatography and Flow Field Flow Fractionation are one of the most common and powerful fractionation techniques. For the determination of the molar mass and size distribution both methods need to be calibrated. The disadvantage is that for exact results a prior knowledge of the chemical structure of the investigated polymers is necessary. Therefore the best method to determine the molar mass and coil size distribution absolutely is to couple the separation device with Multi Angle Laser Light Scattering and a Differential Refractive Index Detector [4, 5].

In this presentation we will present the characterisation of relevant non-ionic, anionic and cationic water soluble polymers in terms of the molar mass and particle size as well as their distributions including the challenges and difficulties during these studies and some potential solutions.

Effect of High Pressure Homogenization on Macromolecular Size and Conformation

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In this paper we study the influence of high pressure homogenization on the molar mass and conformation of waxy barley starch which is a polydisperse ultra-high molar mass biopolymer. The characterization of this material and the influence of the homogenization was made possible through the use of asymmetrical flow field-flow fractionation (AsFIFFF) coupled to refractive index and multi angle light scattering detection. Solutions of the starch were passed through a lab-scale valve homogenizer operating at 60-240 MPa. The treatment had an extensive effect on the size and conformation of the molecules, causing degradation and inducing conformational changes. The extent of degradation was related to the energy dissipation rate during homogenization and, thus, the homogenization pressure.\textsuperscript{1} The conformational changes are reflected in the apparent density of the molecules. By comparing the results for the apparent density from FFF with those obtained through viscosimetry a correlation between density and critical overlap concentration could be established. Furthermore, the hydrodynamic radius of the molecules could be calculated from the elution times.\textsuperscript{2} The results showed that homogenization also induced a profound effect on the shape of the molecules.

Thermal Field-Flow Fractionation of Acrylic Copolymers

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A new thermal field-flow fractionation (ThFFF) method has been developed for the separation and analysis of polyacrylates and acrylic-styrene copolymers. This important class of polymers is commonly used as pressure sensitive adhesives, in coatings and paintings, and as the basis for polyelectrolyte materials. The structure and chemical composition of these polymers play a vital role in the end use of these materials. It is important to accurately analyze and characterize these materials to better understand their structure-property relationships. These polymers have not previously been investigated by ThFFF.

Size exclusion chromatography is commonly used to separate these materials. However, SEC is a size based separation and will not differentiate between copolymers of different chemical composition, or between polymers of different architectures if they are the same size. Thermal FFF, on the other hand, separates analytes according to differences in their thermal diffusion ($D_T$) and normal diffusion ($D$) as expressed in the retention parameter ($\lambda$) equation below.

$$\lambda = \frac{D}{D_T \Delta T}$$

The difference in temperature between the hot and cold walls is denoted as $\Delta T$.

The thermal diffusion process, which is observed in the presence of a temperature gradient, provides ThFFF with the capability to separate polymers on the basis of differences in chemical composition.

The challenge in developing a new analytical method using ThFFF is the identification of a solvent that will retain the polymers of interest. This process is usually done empirically and can be very time consuming. The approach we have taken involves examination of theoretical models proposed by Schimpf and Semenov (1) and Mes et al. (2) and solvent viscosity studies by Kassalainen and Williams (3) to determine the major parameters that affect polymer retention. An additional consideration in this study is the $dn/dc$ value of the polymer-solvent system as a multiangle light scattering-differential refractive index detector combination is used.

This presentation will focus on the selection of an appropriate solvent for ThFFF of PS and PBA, ThFFF analysis of PS-PBA copolymers, and measured $D_T$ values and trends for PS-PBA copolymers and their corresponding homopolymers.

Characterization of Ultra High Molar Mass Poly(Acrylamide) Flocculants using AFFFF-MALS-RI

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The absolute molecular weight distributions of ultra high molar mass poly(acrylamide) (PAM) polymers were measured using asymmetrical flow field-flow fractionation (AFFFF) coupled with multi angle light scattering and refractive index detectors. High molar mass polyacrylamide and their derivatives are extensively used as flocculants and coagulants in mineral and water treating industries. For the ultra-high molar mass PAM samples, it was observed that the mass load onto the separation channel was critical in obtaining a good size separation. In fact it was found that amount of sample injected into the separation channel should be less than (≤) 1µg to ensure separation according to the AFFFF mechanism. However, at such limited sample loads the signal-to-noise ratio (S/N) of the detector responses was very low. To overcome this limitation, a channel with a slot outlet was used to increase the concentration of sample through the detectors. In addition, each sample was injected 10 times and the resulting signals were averaged before calculating the molar mass and radius. Using the above strategies, it was possible to perform separation of PAM having molar mass as high as $10^8$ g/mol and size as big as ~ 250 nm. The weight average molar mass of the highest MW sample was above 18 million Da.
Characterizing protein aggregation and particle formation using Field-Flow Fractionation

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Protein aggregation spans a size range from nanometer to millimeter. Multiple analytical methods have been used to characterize and quantify protein aggregation and particle formation. However, unmet gaps still exist.

Field-flow fractionation (FFF) has the potential to bridge some of these unmet gaps. In addition, FFF could also be used as an orthogonal method against traditional methods such as Size Exclusion Chromatography (SEC) and Analytical Ultracentrifugation (AUC). This presentation will focus on exploring these possibilities.
Flow Field-Flow Fractionation as a Potential Utility to Proteomics

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In proteomics, the analysis of a proteome requires a comprehensive and systematic approach that may include high performance separation methods, mass spectrometric analysis or bioinformatics. The characterization of a protein complex is always complicated since there are frequently a large number of proteins which differ widely in molecular weight (Mw), isoelectric point (pI), and their hydrophilic or hydrophobic natures depending on the cellular states. Recent advances in mass spectrometry have led to remarkable improvements in the ability to characterize complex mixtures of biomolecules in proteomics, however the proper separation of proteins/peptides is still required prior to MS analysis and a high performance protein separation plays a critical role due to the importance of low abundant proteins in relation to biomarker discovery.

Since FlFFF offers a capability to separate proteins in intact states by MW, there is a potential to incorporate it into proteomics research either by on-line hyphenation with mass spectrometry (MS) or off-line combination with nanoflow liquid chromatography-electrospray ionization-tandem MS. In this presentation, discussed are recent efforts to apply FlFFF for pre-fractionation of complicated proteome sample from C. glutamicum, or size fractionation of sub-cellular species such as mitochondria, along with the protein identification of collected fractions utilizing the off-line nanoflow two-dimensional LCLC/MSMS. An initial evaluation of a new high throughput two dimensional protein separation tool will be introduced with a multichannel FlFFF system embedded with isoelectric focusing technique in which fractionation and isolation of proteins can be made by the differences of pI and MW.
Hollow-Fiber Flow Field-Flow Fractionation for the Analysis of Blood Lipoproteins

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Lipoproteins are globular micelle-like particles formed by a core of hydrophobic and neutral lipids, cholesteryl ester, and triacylglycerols, surrounded by a shell of polar lipids and proteins. Lipoprotein profile measurements have become one of the most popular methods to assess lipoprotein abnormalities and the associated coronary artery disease (CAD) risk. It is well known that low-density lipoprotein cholesterol (LDL-C) is the most significant CAD-related risk factor, while high-density lipoprotein cholesterol (HDL-C) exhibits a protective effect. Determination of cholesterol in lipoproteins is routinely performed in clinical analysis [1].

Separation techniques such as LC [2] or CZE were shown to be effective for fast analysis of blood lipoproteins, and they can be considered as valid alternatives to the reference methods. Development of new separation techniques for lipoprotein quantification and characterization can then enhance routine methods for blood lipid profiling. Over a decade, flow FFF (FIFFF) has shown able to separate lipoproteins [3], and hollow-fiber (HF) FIFFF recently showed good separation between HDL and LDL components of human blood [4]. Compared to flat-type, macro-column FIFFF channels, the HF FIFFF channel has the advantage of a reduced channel volume, which is an important aspect if protein characterization wants to be performed after fractionation [5,6]. Analysis time is also reduced, and low-cost channels make it possible disposable analysis formats. These are important features for the development of high-throughput, routine methods to analyze blood lipoproteins.

In this work we extend application of HF FIFFF for the fast separation, quantification, and characterization of lipoproteins in whole human blood serum. The main lipoprotein classes are separated and detected by staining serum samples with lipid-specific dyes. The relative concentration of HDL and LDL components are thereby determined. Lipoprotein size is of particular diagnostic value in lipoprotein analysis, because of the recognized, higher atherogenicity of denser and smaller LDL components. Size and molar mass of lipoprotein fractions are evaluated from experimental retention time values, based on the linear relationship between retention time and particle hydrodynamic radius, and compared to the values obtained by on-line, multi-angle laser scattering (MALS) detection. The method performance is evaluated on serum samples from healthy donors and from dislipidemic patients with high CAD risk. A comparison with results obtained with a commercial, asymmetrical FIFFF-MALS system is performed as well (Eclipse-DAWN HELEOS®, Wyatt Technology Europe GmbH, Germany).

Instrumentation Development in Sedimentation Field Flow Fractionation, from Cell Sorting to Environmental Colloids. from Concepts to Applications and Patents.

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Compared to the diversity observed in liquid chromatography, instrumental development in field flow fractionation remains quite confidential, and limited to very few groups. One of the most sophisticated FFF instrumental set up is related to Sedimentation field flow fractionation and only one device is today commercialized. Our group is now known for his activity in cell sorting using SdFFF systems. However we have chosen, more than 10 years ago for economic and methodological reasons to develop our own system. So far there is very few information available concerning the specificities of the device mostly used for cell sorting and recent patents allow now for more technical and methodological descriptions. In particular “biocompatibility” considerations have directed the choice of given materials as well as methodological attentions.

The objectives of the presentation are to draw the history and the “maturation” processes of instrumental and technical solutions with in mind their applications. Three domains are to be described, the SdFFF column (design, dimensions, set up), volume and geometry compliance control test are described. The second tricky domain resides in the rotating seal technology with the design of systems allowing upgrading the device for Splitt operations. Finally channel basket rotation command and control are described.

Special care where taken account to facilitate routine and replacement maintenance which where included in the design set up. All steps and prototype improvements are shown with the associated application. It appears that a non negligible instrumental “level” or “know how” remains essential for successful separation development in SdFFF.

Figure 1: Schematic presentation of the centrifugation basket with FFF channel

Figure 2: Rotating Seal Design
Hydrodynamic- Acoustic Cell Sorter - \textit{HACS}

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A hydrodynamic-acoustic guide for separating micron-size species is presented. The Hydrodynamic-Acoustic Cell (or particle) Sorter, \textit{HACS}, is composed of a programming acoustic field coupled to a Step-SPLITT channel. Several ultrasonic transducers are placed along the separator. By programming frequency and by adjusting geometrical parameters, very efficient separations of particulate materials are expected. Separations may be performed in function of size and/or acoustic impedance of species.

We present a new scheme for separating particles inside a Step-SPLITT channel by using a juxtaposition of two standing waves with different number of nodes leading to an efficient separation of two species as depicted in figure 1; gravity may be or not used for improving the fractionation process. Numerical calculations of several configurations, number of nodes, number of species, the use of hydrodynamic focusing Step-SPLITT channel, etc, indicates that excellent separations of two or more compounds with very close physicochemical properties like size, density and acoustic impedance should be obtained. Experiments show the capabilities of the \textit{HACS} to be used as a hydrodynamic-acoustic guide of particulate materials flowing in mini-Step-SPLITT channels. The \textit{HACS} should be particularly interesting for biological cells fractionation because the acoustic field is efficient and not intrusive when frequency range is kept between 0.5 and 10 MHz. Macro and \(\mu\)-\textit{HACS} are possible because of the coupling with Step-SPLITT channels.

\textbf{Figure 1 :} A juxtaposition of two standing waves of wavelengths \(\lambda_1\) and \(\lambda_2\) with different number of nodes placed along the thin dimension of the channel may lead to an efficient separation of particles.
Iso-Dielectric Separation for Continuous-Flow Cell Screening

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We introduce the first implementation of a method for the continuous-flow sorting of cells based specifically upon differences in their electrical properties. The method, which we call iso-dielectric separation (IDS), uses the dielectrophoretic (DEP) force in a liquid of spatially varying conductivity to map cell or particle electrical properties to a unique position along the width of a microfluidic channel. The method is analogous to iso-electric focusing, with dielectric properties replacing surface charge as the basis for separation, and can be used to sort cells by electrically distinguishable phenotype.

With IDS, we seek to address some of the limitations inherent in existing DEP separation methods. Current techniques typically sort cells based upon either the magnitude or the sign of the DEP force [1, 2], and are thus either strongly size-dependent or intrinsically binary. The development of DEP field-flow fractionation represented an important advance over previous technologies, using counteracting DEP and gravitational forces to suppress sensitivity to cell volume [3]. However, because DEP-FFF separates particles along the axis of flow, it is not amenable to continuous-flow operation. Additionally, its sensitivity to variations in both cell density and electrical properties reduces the separation’s specificity. Because IDS continuously sorts particles transverse to the direction of flow, it is possible to resolve diverse cell populations with high throughput, independent of the sizes and densities of the constituent cells.

The IDS device consists of a diffusive mixer to generate the conductivity gradient and a separation chamber where electrodes guide cells to the point along the channel width where their electrical properties match those of the fluid (Figure 1). After reaching this iso-dielectric point, the cells flow unobstructed to different outlets for collection. We have tested the device using polystyrene beads, vesicles and yeast. We have demonstrated the simultaneous separation of three types of polystyrene beads based upon surface conductance (Figure 2) and shown that this separation is not possible in a medium with uniform conductivity. We have also demonstrated quantitatively predictable separations of yeast based upon viability. These experiments demonstrate a continuous, specific, and intrinsically analog separation technique, with which we plan to screen engineered cell lines for biomolecule production.

References:
Study Of the Phenotypic Relationship in the IMR-32 Human Neuroblastoma Cell Line by Sedimentation Field Flow Fractionation.

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Since few years, we investigated the implication of SdFFF in cancer research, in particular to study chemical apoptosis or differentiation induction in a cancer cell line [1-5]. Different points can be suggested from 1) the monitoring of the biological event (detection and characterization); 2) the cell sorting of specific subpopulation such as pre-apoptotic, or differentiated cells which could be used as a model and 3) the kinetic of the biological event using both the monitoring and cell separation capacities of SdFFF. These studies, in association with the previous results concerning the human SH-SY5Y clone of the SK-N-SH cell line, demonstrated the interest of SdFFF cell sorting in the goal of this work: study of the differentiation kinetic in the IMR-32 NB cell line. Neuroblastoma (NB) is the most common solid tumor of childhood. Although spontaneous regression can occur in patients less than 1-year old, 70 % of patients over the age of 1 year present high-risk and difficult-to-treat NB. We demonstrated that the neoplastic populations in IMR-32 cell line are extremely heterogeneous and highly variable in their state of differentiation consisting two principal neoplastic cells: 1) neuroblastic or N-type undifferentiated cells; and 2) stromal or S-type differentiated cells. This heterogeneity could affect treatment outcome, in particular the response to apoptosis induced by chemotherapy. Then, it seems relevant to understand the underlying process governing changes in differentiation in order to improve treatment response and NB patient outcome. In this study, we demonstrated that SdFFF permitted the cell sorting of 2 N-phenotypes and the understanding of the IMR-32 cell population dynamism. The first N-phenotype, sorted in PF1, forms a pool of quiescent cells which could produce the second one, sorted in PF3, able to proliferate (BrdU incorporation) and also to differentiate into adherent S-type cells (N-CAM+). Thanks to SdFFF, we isolated N-type cells in PF3 which have characteristics of malignant cells (high expression of nestin) and transiently expressed the PSA-N-CAM which could play a role in the metastatic properties of these cells.

References
Clinical Dielectrophoretic Field Flow Fractionation of Mammalian Cell Mixtures

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Several important diagnostic and therapeutic problems, including the isolation of circulating tumor cells from peripheral blood for prognosis and therapy planning and the isolation of adult stem cells from blood or adipose-derived cell suspensions for stem-cell-based therapies, lack adequate cell isolation capabilities. In previous work, we showed that dielectrophoresis may be applied to greatly increase the discrimination of sedimentation FFF for isolating mammalian cell types and we have demonstrated that it can readily discriminate between tumor and blood cells, for example, without the need to label cells. To be practical for clinical use, however, the technique must be adapted so that it can quickly fractionate tens to hundreds of millions of cells amongst which the target cell subpopulation is but a tiny proportion. This presentation will briefly review dielectrophoretic-FFF and then focus on two limiting phenomena that influence clinically useful cell isolations, namely field-induced aggregation of cells at high cell concentrations and the impact of running buffers on cell physical properties and viability. Data will be presented that illustrates these limitations and strategies for their management will be described in the context of circulating tumor cell and stem cell applications.
Hollow Fiber Flow Field-Flow Fractionation for Sorting of Human Progenitor Cells

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The Hollow Fiber Flow Field-Flow Fractionation (HF FIFFF) [1] is a miniaturized asymmetrical flow FFF sub-technique [2, 3] particularly well suited for cell separation [4]. The system configuration used is specifically designed to reduce sample handling and minimize shear stress on cells in order to maintain viability. It also allows for sample fraction collection. The hollow fiber channel is a tubular polysulfone porous membrane of 200 µm ID (Spectra/Por® in vivo Micro dialysis Hollow Fibers, Spectrum Laboratories, Inc., Rancho Dominguez, CA). A channel volume of about 5 µl and the flow rates used (5 to 20 µl/min elution rate) allow for a reduced dilution of the sample. The volume of sample injected and focused in the Hollow Fiber channel is typically 25µl containing 10⁴ to 10⁶ cells. It is normally possible to collect in about 20µl between 50% and 80% of the cells. We chose KG-1a cells (ATCC, Manassas, VA) as a model for progenitor cells. KG-1a is a primitive human hematopoietic line of myeloid progenitor cells, expressing the CD34 surface antigen, a marker for hematopoietic stem cells. To test the feasibility of discriminating hematopoietic progenitors from other white blood cells with this system, a separation of KG-1a and Jurkat cells, a line derived from human T-cell leukemia expressing CD45 surface antigen, has been performed. The two cell lines have a very similar shape and dimensional distribution, with mean diameters of about 12 µm. However, in our preliminary experiments KG-1a cells showed a slightly longer retention time than Jurkat cells at the same experimental conditions. When a separation of a mixture of the two cell lines was attempted, two fractions were collected after the elution. The fractions were characterized using a fluorescent microscope, after labeling the cells using FITC anti-human CD34 (BD Pharmingen, San Jose, CA) and anti-Human CD45 R-Phycoerythrin (Caltag Laboratories, Invitrogen Corporation, Carlsbad, CA). The low dilution of the sample during the analysis greatly facilitated the labeling and staining procedure. The fluorescent antibodies were directly added to the collected fraction vials, without any additional sample preparation. In the photomicrograph the KG-1a cells are visible by the green fluorescent emission of the FITC and the Jurkat cells by the red fluorescent emission of R-Phycoerythrin. Compared to the original sample, the fraction at lower retention time shows enrichment in Jurkat cells, while the second fraction is enriched in KG-1a cells. We believe this to be evidence of the separative performance for this system.

References

Laser-Photophoretic Migration and Separation of Human Blood Cells

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Gradient force of laser beam has been widely studied and already been utilized for trapping, levitation, or manipulation of micrometer-sized particles or biological cells in liquid media. On the other hand, scattering force of laser is less developed as a force for the migration or the manipulation of microparticles, although Ashkin had reported such a force in 1970[1]. As the scattering force of the laser light arises from scattering and/or absorption of the light by the particle, laser-photophoresis has an advantage of being able to migrate any particle, which has refractive index different from that of surrounding medium. We have proposed a laser-photophoresis of particles, which is a unique technique to migrate and to characterize particles in liquid and developed fundamental studies on the photophoresis of various particles in liquid media[2-6]. In this study we demonstrated the laser-photophoretic separation and characterization of human blood cells.

The photophoretic migration behaviors of red blood cells (RBCs), white blood cells (WBCs), and platelets (PLs) in isotonic aqueous solution were examined by irradiating Nd:YAG laser (532 nm, 0.2 to 2.0W, beam radius: 30 to 100 µm). It was observed that the photophoretic velocity of PLs (ca. 1 µm) was smaller than that of WBCs (5 to 10 µm) due to the size effect. While the photophoretic velocity of RBCs is more than ten times greater than that of WBCs, although the average size of WBCs is comparable or lager than that of RBCs (6 to 8 µm, 2 µm thick). The higher photophoretic velocity of RBCs is considered to be due to the absorption of 532 nm light by RBCs, namely, RBCs receive the momentum of the light by both scattering and absorption, while WBCs receive the momentum of the light by only scattering. These observations indicate that the fractionation of blood cells in whole blood could be achieved by using laser-photophoresis in a simple micro-flow system.

In a flow system, the Nd:YAG laser irradiated perpendicular to the bulk flow direction and change in the stream lines of blood cells due to the photopheretic migration were observed. The deflection in the stream lines of the respective cells were proportional to the photophoretic velocities. By using a simple double-Y type micro-channel device, the separation of RBCs from other blood cells was achieved.

It was also observed that the photophoretic velocity of RBCs strongly depends upon the orientation of RBCs. RBCs tend to migrate their disk plane parallel to the migration direction. The migration velocity when the laser irradiates parallel to the RBC disk plane is about 5 times larger than that when the laser irradiates perpendicular to the RBC disk plane.

References
Natural colloids and metals: fractionation and speciation based on Asymmetrical Flow Field Flow Fractionation

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The term “colloids” in environment refers to a wide variety of macromolecules (viruses, biopolymers, humics, inorganic particles…). Natural colloids are known to play a major role in environmental processes and particularly in transfer, bioavailability and cycle of contaminants (organic pollutants, heavy metals, metalloids…). In soils, colloids are mainly composed of organic substances such as humic and fulvic acids and inorganic particles.

This work shows the potentiality of online and off-line Asymmetrical Flow Field Flow Fractionation (As-Fl-FFF)-based hyphenated techniques associated to a multi-detection approach to obtain size repartition of environmental particles and reach physical as well as chemical metal distribution on colloids.

The multi-detection approach is based on the hyphenation of As-Fl-FFF with UV, Multi Angle Laser Light Scattering (MALLS) and Inductively Coupled Plasma Mass Spectrometry (ICP MS). Hyphenation also involves chromatographic separation and selective detection in order to perform speciation analysis. The combination of AsFIFFF-UV-MALLS-ICP-MS and speciation analysis is useful to determine the actual environmental impact of trace elements such as arsenic, selenium or tin, which have several organic and inorganic species with various physico-chemical properties and toxicities.

The analytical approach consists in a two-step methodology. First, AsFIFFF-UV-MALLS-ICP-MS directly gives information on natural particle sizes with gyration radius (Rg) whereas ICPMS informs on distribution of trace elements according to colloidal separation. Complementary, ICPMS permits to reach qualitative and quantitative data about metals linked to colloids according to colloidal nature. Secondly, fraction collection is performed according to the trace element distribution previously determined. Then, each colloidal fraction is analysed in order to speciate the elements of interest. This second step requires high-sensitive and selective analytical method, in agreement with environmental concentration levels.

In this presentation, this two-step methodology is illustrated through the example of organotin compounds (OTC). OTC are considered as among the most toxic chemical pollutants, being harmful for terrestrial and aquatic life since some ng(Sn)/L. In the present case, speciation analysis is performed by Headspace - Solid Phase Micro Extraction (HS-SPME) - Gas Chromatography (GC) - tin specific detectors (Pulsed Flame Photometric Detection - PFPD). The current analytical challenge in this case is to be able to reach extremely low limits of detection (sub ng/L) in very complex matrix with little amount of sample. OTC physical and chemical speciation analysis is performed on different environmental samples, such as soil solutions. The results obtained show that species distribution involving butyl- and phenyltins widely depends on the considered OTC and fraction (organic or inorganic colloids, dissolved phase). This two-step methodology is found to provide new environmental information, especially about complexation between metal species and colloidal ligands.
New Tools for Optimization of Channel Geometry and Method Development in Asymmetrical Flow-Field Flow Fractionation (AF4)

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Asymmetrical Flow-FFF (AF4) is currently the most widely used FFF technique with a significant installed base both in industry and academia worldwide. AF4 is a variation of Flow-FFF which has been originally developed by Karl-Gustav Wahlund [1] who also published the necessary modifications to the retention equations of symmetrical Flow-FFF. Guidelines about typical channel dimensions and possible ways to construct a channel exist [2] as well as guidelines how to choose appropriate conditions for working out a separation method, but they are either empirical, or not straightforward to use and limited in their scope.

Here a new evaluation is given by using simulated chromatograms, taken out of a finite element calculation of the flows in the separation channel. For every volume element the calculation includes the strict FFF- theory [1]. The resulting simulated chromatogram is valid and shows the exact influence of experimental parameters (channel dimensions, variable flow rates, sample concentration, membrane permeability, etc.) on elution time, peak size and shape. The simulation tool quickly helps to find out inconsistencies in an experiment, and is an efficient tool to optimize experiments and data evaluation. This is demonstrated for a BSA and a dextrane sample. Besides that the simulation gives new access to the FFF-channel design. Systematic theoretical studies and verification with experiments have resulted in new channel geometries that are quite different from the traditional design.

Macrotransport analysis of thermal field-flowfractionation

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Thermal field-flow fractionation (ThFFF) processes are analyzed using the general principles of “Macrotransport Processes” [1]. This is a broadly-based generic scheme for analyzing highly complex problems in generalized Taylor dispersion theory, of which FFF is an example. Though the computational scheme has been available for some time, and possesses significant potential for analyzing highly complex flow-diffusion-heat transfer-chemical reaction problems in a straightforward “cook-book”-like fashion, its principles and advantages over other schemes appear to be relative unknown to the FFF community as revealed by a search of the literature. The scheme will be applied to study ThFFF processes involving complications resulting from dependence of the system’s physics on: (i) distortion of the Poiseuille velocity field owing to the fluid’s temperature-dependent viscosity; (ii) wall effects; (iii) apparatus orientation relative to gravity; (iv) temperature-dependence of the fluid’s phenomenological transport coefficients, etc. It will be pointed out how all these factors could, in principle be addressed simultaneously, using the generic solution scheme of “Macrotransport Processes.” By simultaneous is meant without treating each effect separately and then simply adding the separate effects linearly — which would be highly approximate, at best. Explicit results will be presented in several special cases and compared against competitive computational schemes and experiment.

References

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Computational fluid dynamics (CFD) simulations has been used to predict the complex flow pattern caused by the presence of an outlet stream splitting device in a field-flow fractionation (FFF) channel for asymmetrical flow FFF. The stream splitter was a simple rectangular hole drilled in the depletion wall situated just a short distance upstream from the regular channel outlet. Through the stream splitting hole a major part of the carrier was caused to leave, while supposedly causing the very thin concentrated sample layer to exit the channel by the regular outlet stream connected to the detector. This arrangement is expected to generate a detection sensitivity enhancement. The experimental effect of the outlet stream splitting was measured in terms of the total method sensitivity (peak height), retention time, and resolution. A circular stream splitting hole was also evaluated, but only experimentally. Depending on the geometry of the hole, and the magnitudes of the two splitted outlet flow rates, the experimental results were influenced differently. With optimized flow rates a clearly useful sensitivity enhancement was obtained. It ranged 1.8 – 15. However, its efficiency was only 26 % - 75 %. Moreover, the sample components had unexpectedly high retention times and a decrease in resolution was observed. The CFD simulations did explain these shortcomings. Their origin is that the parabolic flow velocity profile and the stream-line pattern of the carrier channel flow, in the vicinity of the splitting hole, is greatly disturbed by the presence of this hole.
The Steric Correction on the Potential Barrier Field Flow Chromatography

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The steric transition region of field-flow fractionation (FFF) is described as that part of a fractogram, in which normal FFF undergoes a transition to steric FFF by virtue of increasing particle diameter. As the particle size increases, the size-based effects increase and the particle velocity goes through a minimum (called the inversion point) and begins increasing.

During the analysis of a colloidal sample by sedimentation field-flow fractionation (SdFFF), it is of vital importance to know if the particles are eluted in the normal or the steric mode. Consequently, the estimation of the transition point is of great necessity, since this can lead to the selection of the appropriate experimental conditions for the sample analysis.

In the present work a simple methodology for the estimation of the inversion point is introduced, based on the theoretical and the experimental values of retention ratio. The steric effect on the distance of the particles from the accumulation work and the experimentally estimated potential energy of interaction can be evaluated. Its value can be estimated by a specific value of ionic strength which is sufficient to disable the steric effect. The experimental results are in accord with those given in literature.
Spectroscopic Approach to the FFF Steric Inversion Problem

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The characterization of polydisperse particle mixtures that span the one micrometer size has been a long standing problem for many particle sizing techniques. Optical microscopy, photon correlation spectroscopy, Fraunhofer diffraction, normal- and steric-mode field-flow fractionation (FFF) all have limits around this size region.

For the FFF techniques, a polydisperse sample mixture possessing particles ranging in size from 0.3 to 3 \(\mu\text{m}\) will experience dual mode separation as the smaller particles undergo normal mode separation (small particles elute first) while the larger particles undergo steric mode separation (large particles elute first). Since both separation mechanisms are simultaneously taking place, two different particle sizes can coelute at any given time. Data interpretation is difficult, if not impossible, under these circumstances. It has been demonstrated that the transition between normal and steric modes can be shifted by using different flow velocities and field strengths. However, these shifts in the steric inversion diameter are usually insufficient to address the 0.3 to 3 \(\mu\text{m}\) (or 0.1 to 3 \(\mu\text{m}\), etc.) sample mixture. Consequently, the application of the FFF techniques to polydisperse particle samples that span the 1 \(\mu\text{m}\) size has been restricted.

We have resolved the problem of steric inversion by using a spectroscopic approach. In this case, the FFF detector is a light scattering-based sensor that sizes and counts particles as they cross a laser beam. The detected photovoltage produced by single particle light scattering is registered in particle diameter channels or bins generated by calibration of the sensor response (photovoltage) using a set of size certified particle standards. The size information is stored in one of fifteen size bins. Different numerical values are assigned to each bin depending on the desired size resolution, e.g., each bin can differ by 0.1 \(\mu\text{m}\), 0.3 \(\mu\text{m}\), etc. Since the sizes of individual particles are measured and the information stored in separate bins, it is possible to obtain precise size measurements of coeluting particles. In addition, as the number of particles of each size is counted, number-based particle size distributions can be obtained. This presentation will describe the principles of this sensor and demonstrate the ability of flow FFF with dual sensors to address steric inversion.
Performances of differential field-flow fractionation as an analytical technique: Investigation on precision in differential sedimentation FFF

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Generally, the characterization of the sample components analyzed by field-flow fractionation (FFF) relies on a relationship between the experimentally measured retention time and the analyte parameter of interest (for instance, particle size or molar mass). This relationship derives from a retention model or from an appropriate calibration procedure. Another possibility of exploiting FFF consists in determining parameters which rely, not on a single FFF retention experiment, but on retention in, at least, two FFF runs performed not with a single identical analyte, but on an analyte which has undergone a modification. The comparison between the retention data in the two runs allows to get information on that modification. This is the basis of the concept of differential FFF.

This approach has been used in the past for the investigation of the equilibrium and kinetic parameters of the coating adsorbed on bare colloidal particles as well as of the equilibrium constants of interactions taking place at their surface (for instance, antigen-antibody interactions).

A key issue in this differential FFF approach concerns the limit of detection, i.e. the minimum amount of adsorbate per particle that can be determined at a given probability level (e.g. 95%). This limit relies heavily on the precision of the individual retention measurements.

In this study, two kinds of random errors affecting the determination of retention times were considered: those arising from the fluctuations of the operating parameters (temperature, flow-rate, field strength) and those arising from the detector noise, which affect the precision with which the retention time is determined from an experimental fractogram. The underlying theory for these two kinds of errors is developed. The errors are computed for the case of the investigation of the adsorption of IgG on bare polystyrene submicron particles by sedimentation FFF and compared with experiments. The errors arising from detector noise appear to be the main source limiting the precision. The detection limits and confidence intervals of the adsorbed mass uptake are determined as a function of experimental quantities (retention ratio, injected quantity, baseline noise, void volume relative error). They are found, both theoretically and experimentally, to depend on the square root of the injected sample concentration, for a constant injection volume.
High-Temperature Flow Field-Flow Fractionation – A New FFF Technique for Polymer Characterization

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High Temperature Flow Field-Flow Fractionation (HTFFF) coupled to infrared (IR), light scattering (LS), refractive index (RI) and viscometry (Visc.) is introduced as a new FFF technique for the characterization of high and ultrahigh molecular weight polymers at elevated temperatures.

FFF separations at higher temperatures provide a variety of new, so far undiscovered, applications in the area of polymers and particles. Because of the non-availability of commercial HTFFF systems, up to now the majority of the FFF applications published in the pertinent literature was done under room temperature conditions. Nevertheless, some interesting work was done in the past already using Thermal and Flow Field-Flow Fractionation to generate applications at high temperatures.

First basic work was done by Giddings and Miller \cite{1}, which used a prototype-based modified symmetrical Flow FFF for separations at elevated temperatures. The system was able to work with Xylene at temperatures up to 140°C for separation of polystyrenes. Unfortunately, no further publications followed up this interesting work. Main limitation of this presented set-up was the membrane, which was suitable only for limited solvent systems and which showed restricted live time at the used temperatures.

More extensive developments in this area have been done by Brimhall, Pasti, Melucci and others \cite{2-7}. These groups used commercially available Thermal FFF systems which were modified and applied to the separation of PE and PP as well as PE and PS. This fundamental work for the first time showed the broad possibilities of high temperature separations using FFF. Unfortunately, the technical set-up using high temperature Thermal FFF is complex and additionally, low thermal diffusion coefficients are limiting the application range and restricting the possible solvents.

Based on this fundamental work done in the past a new High Temperature Asymmetric Flow FFF (HTFFF) was developed. The technology and different polymer applications, such as polyolefins, etc., are presented. The differences between HTFFF and HTGPC when applied to the characterization of high, ultrahigh and branched macromolecules are highlighted.

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The development of a magnetophoresis instrument for the production of biological labels

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Fluorescence-based biological labels are limited in number by the variety of dyes presently available. Utilizing the magnetic properties of microparticles offers the potential for orders of magnitude more labels, and it is for this reason that we are developing a magnetophoresis instrument for the production of biological labels for in-vitro studies.

Here we will discuss the constituent elements of our magnetophoresis instrument, namely the microparticles, microfluidic flow chamber, and magnetic field source.

For in-vitro studies, the requirement that nanoparticles be used is somewhat relaxed, thereby permitting larger diameter (and hence larger magnetic moment) particles. We have run proof of principle experiments with commercial ferromagnetic and superparamagnetic microparticles and also with novel paramagnetic iron particles. Particle diameters range from 4 to 50 microns (dependent on sample used), with corresponding magnetic moments of the order of $10^{-13}$ Am$^2$.

The laser-cut microfluidic chamber has a single sample inlet and two sheath inlets (from the same supply). In the sorting region of the chamber we have essentially laminar flow, though the aspect ratio of the chamber introduces some hydrodynamic instability that we have corrected [1]. The sorted microparticles are then extracted with the aid of syringe drives into multiple collection tubes – our first chamber had 8 outlets, while the latest version has 25. We will discuss the reliability and reproducibility of the sorting process.

The microparticles are sorted (according to their magnetic moment) [2] by flowing them through the chamber in the presence of a magnetic field. To supply the magnetic field we have used either a simple block magnet or a magnet quadrupole; the former generates a non-linear field gradient while the latter has a linear field gradient. The form of the field and field gradient dictates the trajectory of the microparticles through the chamber. We will compare the measured distributions of sorted microparticles with modeled data.

References

Quadrupole Magnetic Field-Flow Fractionation: A Novel Technique for the Characterization of Magnetic Nanoparticles

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Magnetic Field-Flow Fractionation (MgFFF) [1-3] is a relatively new form of FFF that has the potential of quantifying the distribution of magnetic material in a nano-sized particulate sample. The geometrical design of the system differs from that of other typical forms of FFF in that it exploits the radially symmetric field of a quadrupole magnet using a helical channel.

Magnetic nanoparticles are finding many uses in biotechnology and medicine. They are used for cell labeling and separation, drug targeting, and hyperthermia, for example. For such purposes, they are generally made of a core of magnetic material coated with a biocompatible material such as dextran. This helps to both stabilize them and disperse them in suspension, and provides a substrate for attachment of antibodies. They are generally superparamagnetic in which case they become magnetized only in a magnetic field and lose their magnetization when the field is removed. This feature makes them particularly useful for their biomedical applications.

The magnetic field induces magnetization of the nanoparticles which then experience a force $F_m$ due to their interaction with the field gradient:

$$F_m = V_m M \nabla B$$

in which $V_m$ is the volume of magnetic material in the particle core, $M$ is its magnetization, and $\nabla B$ is the gradient in field $B$. The coating material generally has a negligible interaction with the field. As in sedimentation FFF, the strength of particle interaction with the field and, therefore, particle retention are dependent on volume of material. The selectivity with respect to core diameter is therefore close to 3. As in sedimentation FFF, elution of polydisperse samples therefore demands programmed decay of field strength. The power supply to the quadrupole electromagnet is controlled by a computer, and the system can provide any desired field decay. With assumed dependence of core magnetization on applied field strength, data reduction yields a distribution of magnetic core diameter.

Examples will be shown of the separation and characterization of magnetic nanoparticles (kindly supplied by BD Pharmingen), of the type used to prepare immunospecific labels for magnetic separation of biological cells.

References


Separation of Micron-Size Species by using the Step-SPLITT Fractionation Channel

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Step-SPLITT fractionation channel is a new device which eliminates the drawbacks, related to splitter imperfections and misalignments, like the non-specific crossover. Moreover, splitters make difficult setting up micro and mini-channels and extending SPLITT fractionation to separations of more than two compounds. The channel is composed of inlet and outlet pre-chambers placed at a different level than the main channel as depicted in figure 1. In this design, one of the inlet and outlet flows passes from the pre-chambers either to the main channel or from the main channel to the outlet pre-chamber through a slot which has the same channel breadth, the thickness being of comparable to that of the channel. This new architecture, patented by our team, allows us to built SPLITT-like channels of any dimension, including microfluidic and very big ones. This new design allows also manufacturing channels provided with multi-inlet and multi-outlet ports.

We shall present the channel architecture and its advantages compared to classical ones, flow field FLUENT simulations, separations and enrichments of species from different mixtures. Experiments have been performed in two different Step-SPLITT devices, one of those being a mini-channel. Mixtures used are on the one hand, particles of 5-8 and 5-10 $\mu$m, on the other hand, a mixture of macrophages infected by the parasite \textit{Leishmania amazonensis} and healthy macrophages. We shall also display \textit{in situ} 3D visualization, obtained by digital holographic microscopy, of particles, red blood cells and phospholipid vesicles flowing through the channel. We will underline the possibility of separating phospholipid vesicles of different sizes by using the lift operation mode.

![Figure 1 Schematic view of the Step-SPLITT fractionation channel](image)
Optimization of Cyclical Electrical Field Flow Fractionation

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In CyElFFF, the retention of particles is highest when the retention parameter is very small or when it is very large. When the retention parameter is very small (\( \lambda_0<<1 \)), which is our case of interest, particles elute in two segments causing an unwanted early peak. To eliminate this early peak, a particle relaxation technique is used in this work: The carrier flow is stopped approximately 8 seconds after injecting the sample particles (for flow rate of 1ml/min) so that particles barely enter the FFF channel and a DC field is applied to force the particles towards one of the walls. As the DC field exponentially decreases with the time, the potential is applied after the particles enter the channel. After stopping the flow for 100 seconds cyclical fields are super imposed on the DC field and carrier flow is started. The DC field is maintained throughout the experiment, otherwise particles will move away from the wall because of discharging effects.

**Effect of offset voltage on Elution Time:** Figure 1, shows a plot of elution time as a function of offset voltage. This plot is obtained by applying a square wave of 2Vpp and 10 Hz frequency on 209 nanometer polystyrene particles. From the plot, we note that the elution time increases very little until the offset voltage is increased to about 1 V. After this point, the elution time increases rapidly with any increase in offset voltage. When the offset voltage is below 1 V, the influence of offset voltage on the cyclical fields is negligible. As the offset voltage is increased above 1 V, we leave the cyclical domain and begin to enter the normal electrical FFF domain, but with a voltage perturbation on top of the applied field. The retention ratio obtained in these experiments was as high as 20 where as the maximum retention ratio obtained previously was only 5.

![Figure 1: Plot of Elution time Vs Offset Voltage](image)
Optimization of Particle Separation in Different Elution Modes in Field-Flow Fractionation

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Field-flow fractionation (FFF) represents a family of versatile elution techniques suited for separation and characterization of macromolecules and particles. Separation results from combination of a non-uniform flow velocity profile of a carrier liquid and a non-uniform transverse concentration profile of an analyte caused by the action of a force field. The field, oriented perpendicularly to the direction of the flow, forms a specific concentration distribution of the analyte inside the channel. Because of the flow velocity profile, different analytes are displaced along the channel with different mean velocities, and thus their separation is reached.

Based on the magnitude of the acting field, on the properties of the analyte and, in some cases, on the flow rate of the carrier liquid, different elution modes are observed. The differences among them arise from the course and magnitude of the resulting force acting on the analyte. They differ in the type of the resulting concentration profiles of the analyte inside the fractionation channel. Three types of the concentration profiles can be derived from the general transport equation and three basic elution modes were described.

Determination of the elution mode is very important for evaluation of the retention data because various elution modes are described by different retention equations, which leads to different values of calculated properties of analytes. An experimental procedure for determination of the elution mode based on changes of the force applied and the flow rate is described. Regarding the force field programming in GFFF, we focused on two topics: changes of the properties of carrier liquids (density, viscosity) and influencing lift forces achieved by changing the flow velocity of the carrier liquid inside the channel. We have predicted (1) and described (2) the experimental conditions applicable to force field programming in the case of separations of silica gel particles (2) and starch granules (3) by GFFF.

Force field programming in gravitational field-flow fractionation based on modulation of hydrodynamic lift forces has been optimized. The flow-rate control of hydrodynamic lift forces has been accomplished by programmable pump (4) and the flow-velocity rate control of hydrodynamic lift forces has been accomplished by using non-constant cross-sections of the fractionation channels (5). Several flow-rate gradients induced by programmed pumping (step gradients, linear gradients, parabolic, and combined ones) or by the changing profile of the fractionation channel (channels with non-constant height or width) have been used for separation of model mixtures of silica gel particles or latexes and isolated starch granules. The optimized conditions were used for characterization of distribution of starch granules isolated from different barley varieties.

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Abstracts for
Poster Presentations
Size Determination of Nano-Sphere by Field-Flow Fractionation and Pulsed Field Gradient Nuclear Magnetic Resonance

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A powerful tool is required for size determination of nano-particle in solution on each chemical and biological field, for example: the drug carrier with a diameter from ca. 10 nm to 200 nm has been used as drug targeting materials, while the sphere of which diameter is smaller than 5 nm is excreted through renal filtration, therefore appropriate determination of the nano-sized materials is necessary. Utilizing the dynamic light scattering (DLS) and the pulsed field gradient nuclear magnetic resonance (PFG-NMR) spectroscopic method, averaged sizes of a nano-particle could be estimated with rough information of the size distribution. However, the precise measurement of the size distribution is important in order to obtain the correct physical properties for those nano-particle. Furthermore, the truthful size distribution is necessary to compare with those two different averaged sizes determined by DLS and PFG-NMR methods. Field flow fractionation system is one of the solutions for such a problem. However, there are other two different problems to measure the precise size and size distribution. One is the aggregation during the sample focusing, and the other is the overestimation of the sizes of nano-particle because of bound salts or surfactants.

In this work, we succeeded in the determination of a hard-core sphere particle in aqueous solution that has approximately 30 nm diameter size by PFG-NMR. Using this result, we clarified the effects of those two problems on precise size determination by the FFF - multi angle light scattering (FFF-MALS) methods.

![Figure 1. Example of PFG-NMR attenuation plots for 30 nm diameter particle size standard in water when \( \Delta = 50 \) ms and \( \delta = 1 \) ms were kept as constant.](image1)

![Figure 2. Example of the size distribution for 30 nm diameter particle size standard in 0.36 mg/ml sodium dodecyl sulfate aqueous solution.](image2)
Fractionation and Characterization of Gold Nanoparticles in Aqueous Solution: Asymmetric-Flow Field-Flow Fractionation with MALS, DLS and UV-Vis Detection

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Nanoparticles (NPs) have demonstrated great promise in biomedical applications, such as targeted therapeutics, diagnostics and image contrast enhancement. The standardization of physical characterization protocols for NPs is critical for their eventual approval and use in clinical settings, and for the development of reliable nanosize reference materials.

Asymmetric-flow field-flow fractionation (A-FFF)[1,2] separates constituents based on hydrodynamic size, and is emerging as a powerful tool to obtain high-resolution information on the composition, stability, size, molecular weight, and frequency of dimer, trimer and higher order aggregates in unfractionated NP solutions.

In the present work we employ a commercial A-FFF system customized with MALS, DLS, DRI and DAD detectors, to establish fundamental protocols for the characterization of citrate-stabilized gold nanoparticles (GNPs). These protocols are being applied in the development of new gold-based nanosize reference materials.

We have optimized the experimental conditions by controlling key parameters, such injection concentration, mobile phase composition, membrane pore size and material, and ratio of channel-to-cross flow rates. Individual GNP components were separated chromatographically by A-FFF from multi-component GNP mixtures. We report on the results of these studies and their implications for biomedical research involving GNP-based materials.

References

Physicochemical Study of the Differently Degraded Sodium Hyaluronate (Naha) by Flow Field-Flow Fractionation and On-Line Multiangle Light Scattering

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Sodium Hyaluronate (NaHA) is water soluble, ultrahigh molecular weight linear polysaccharides composed of disaccharide repeating unit (D-gluconic acid and N-acetyl-D-glucosamine). NaHA has been found in body tissues, synovial fluid, the vitreous humor, the umbilical cord, and it is known to protect cells and tissue from the invasion of bacteria in biological system. Since NaHA has numerous practical applications including ophthalmic surgery, cosmetics, the treatment of knee joint disease, and etc. as in their intact or degraded forms, the refinement processes of NaHA including degradation using a chemical or physical means are necessary for desired pharmaceutical applications. In this study, the on-line frit inlet asymmetrical flow field-flow fractionation (FI-AFIFFF) /multi-angle light scattering (MALS) was utilized to study the influence of the gamma radiation degradation and the refinement process on molecular weight distribution and structure of NaHA. It showed that a simple refinement process provided different fractions of NaHA’s (in terms of MW and viscosity) without changing the conformation, however the gamma radiation degradation induced a significant change in MWD and in conformation of NaHA structures.
Application of Gravitational Field Flow Fractionation and VIEEW™ Apparatus to Characterization of Irregular Shape Particles

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Gravitational Field Flow Fractionation (G-FFF) is widely used for characterization of different particles. However, all of the equations for calculation of particle size are suitable for the regular or near spherical shape particles and characterization of irregular shape ones is difficult. For these particles, a well-known equivalent diameter i.e. classical aerodynamic diameter is defined [1]. For a particle of arbitrary density $\rho_p$ the shape factor $\chi_p$ takes into account the influence of the particle shape on the settling velocity (equation 1). For the flake-like irregular shape particles, supposing the height of the particle is homogenous for all particles, $d_{ve}$ (the volume equivalent diameter) can be calculated via equation 2:

$$d_{aero} \approx d_{ve} \sqrt[3]{\frac{\rho_p}{\rho_0 \chi_p}} \quad (1)$$

$$d_{ve} = \sqrt[3]{\frac{6S_h}{\pi f n}} \quad (2)$$

where $S ($µm²$)$ and $h ($µm$)$ are the surface and the height of the irregular shape particles respectively, $f$ is the magnification of SEM photo and $n$ is the particle number. $S$ and $h$ can be obtained by the Video Image Enhanced Evaluation of Weathering (VIEEW™) system and Scanning Electron Microscopy (SEM) respectively. VIEEW™ apparatus, including an image acquisition software unit and separate image processing/analysis software, is originally used as an inspection system for some defect types on coatings systems. In this software the surface of the point area is measured in mm²

In this study a polydispers graphite sample (in shape and in size), with a mean particle size equal to 19± 0.5 µm, is characterized via G-FFF and the results are compared to those from laser diffraction particle size analyzer (LDpsa). SEM photos of crude sample show that a large part of particles are in irregular shape.

From G-FFF elution profile the fractions were collected at different elution times and investigated by SEM and VIEEW™ system. Using equations 1 and 2 the values of $d_{aero}$ were calculated. The results show that $d_{aero}$ corresponding to the mean particle size is equal to 17.8 ± 0.4 µm being in satisfactory agreement with the value obtained by LDpsa.

It can be concluded that a combination of G-FFF, SEM and VIEEW™ results can be used as a satisfactory method to obtain the particle size analysis of the particles of irregular shape.

**Keyword:** G-FFF, VIEEW™, shape factor, SEM

Blood plasma volume expanders

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Blood plasma volume expanders are used as a substitute for human blood. In the past, isotonic saline solution has been used to compensate for blood loss in surgery or accidents. In addition to severe disadvantages, only blood loss up to 200 mL could be compensated. In more recent years, polysaccharides like hydroxyethylstarch (HES) and dextran have been used because of their longer shelf-life and lower risk of infections compared to human blood. In Germany, HES is widely applied. Disadvantages of HES are anaphylactic reactions, when a high molar mass tail is present in the molar mass distribution[1]. Also accumulation in tissue and organs of rats and rabbits could be proven. Voluntary tests on human patients also showed deposits in kidney, lung and skin, the latter causing persistent itching resistant to common therapy[2]. The deposits could be linked to the ether-bond of HES, which cannot be split by body-inherent enzymes. Therefore, as a new approach, acetylstarch (AS) has been introduced, since it can be degraded by esterases and glucosidases (for the amylopectin backbone) leaving no deposits in tissue or organs[3]. Therefore, shelf-life of randomly substituted AS is short[3]. O-2-substituted AS has a longer shelf-life because of the sterically hindered attack at C-2. As a new approach, O-2-AS has been synthesized and characterized in terms of molar mass and particle size distribution[4,5]. Also the shelf-life has been determined by viscometry, NMR, UV/VIS and SEC/MALLS/DRI measurements[4,5].

In conclusion, a new blood plasma volume expander could be synthesized with a suitable shelf-life and a good biocompatibility.

Separation and Quantitation of Silver Nanoparticles using Sedimentation Field Flow Fractionation

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Sedimentation field-flow fractionation (SdFFF) provides a mass-based separation, and thus a size-based separation for particles of uniform density.

In this study, SdFFF was employed for separation and determination of size distributions of silver nanoparticles of about 100 nm in diameters. Various experimental parameters were varied to find an optimum SdFFF condition for separation and analysis of silver nanoparticles. The field and/or flow programming were also tested to improve the resolution.

The use of pure water as the carrier liquid did not produce a normal elution profile, probably due to charge interactions between particles themselves and between the particles and the channel wall. Water with 0.1% FL-70 was chosen as the dispersing medium and also as the carrier for SdFFF analysis of silver nanoparticles.

The relative abundances of each population in partially resolved binary mixtures of silver nanoparticles were determined by mathematically de-convoluting the SdFFF fractograms.
An Approach to Characterize Industrially Important Polyacrylate Mixtures by Thermal Field-Flow Fractionation

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The optimal performance of polymers used for industrial application requires a thorough characterization including determination of molar mass, size and chemical composition. These applications often involve a mixture of polymers rather than a single polymer; therefore emphasis needs to be placed on determining chemical heterogeneity in mixtures.

Size exclusion chromatography (SEC) is a commonly used technique for determining molar mass. However, SEC separates polymers by their hydrodynamic size; thereby limiting its usefulness for the analysis of polymer mixtures containing similar sized but different composition species1. In addition, SEC is not applicable to ultrahigh molecular weight polymers because of shear degradation and suffers sample loss due to adsorption to the column packing material2. Thermal field-flow fractionation (ThFFF) is an alternate and complementary technique to SEC. The former utilizes a temperature gradient acting perpendicular to the carrier flow3 and an open channel to separate polymers. ThFFF is able to separate polymers based on size (similar to SEC) and has the additional capability to differentiate by chemical composition due to differing thermal diffusion coefficients, DT, of the components. Thermal diffusion, which has been demonstrated to be specific for each polymer-solvent system, opens up possibilities for achieving separations of complex polymer mixtures. The open FFF channel design also allows for the analysis of ultrahigh molecular weight polymers and microgels.

This presentation will describe the use of ThFFF to characterize the industrially important class of acrylate polymer mixtures and microgels.

References:

Sedimentation Field-Flow Fractionation of Polystyrene Beads Coated with Iga: Carrier Composition Effects on Complex Characterization.

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Sedimentation FFF is a sensitive method for measuring the mass of an adsorbed layer on colloidal particles and it is able to evaluate surface concentrations of immunoglobulins or antibodies (Igs) adsorbed to polystyrene latex particles [1-2].

Immunoglobulin A (IgA) is the main immunoglobulin in mucous secretions, including tears, saliva and colostrum. IgA believed to play an important role in defense mechanisms against infections and because it is resistant to degradation by enzymes, secretory IgA provides protection against microbes proliferating in body secretions, especially those of the digestive and respiratory tracts [3].

Binding of Igs onto a hydrophilic surfaces lies at the center of a multitude of biological, biomedical and biotechnological application. Chromatographic supports, membranes and latex particles are only few examples of substrates on which immunoproteins may be adsorbed. Immobilization of the Igs may be due either by physical adsorption or by covalent binding to functionalized surface groups. Appropriate conditions for binding are as varied as the type of molecules concerned and their chemical binding properties. IgA show a strong tendency to adsorb on polystyrene (PS), under several different chemical-physical conditions.

In this work the mass of IgA adsorbed onto PS microsphere standard has been determined from SdFFF fractograms obtained by varying the mobile phase composition. The compatibility of the SdFFF channel with several buffers used as carriers was proven, in particular, density, chemical composition, its pH and its ionic content, have been considered as experimental variables. Their ability to correctly fractionate the PS and the PS-IgA complex has been tested on two monodispersed polystyrene latex microbeads samples.

The surface reactivity of the complex PS-IgA has been verified by preparing the ternary complex PS-IgA-antiIgA (specific antigen-antibody reaction) and by monitoring the increase of mass by SdFFF. The negative control was the reaction between the complex PS-IgA and anti IgE, which did not occurred as proven by the registered fractograms.


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Perfluorocarbon (PFC) emulsions are used in drug delivery and as temporary synthetic carriers to deliver oxygen to tissues. Size characterization of PFC in blood is complicated by the presence of different blood components such as cells, proteins and small molecules.

Characterization of blood samples and emulsions in a single run is challenging. Blood cells that are considerably (about 5-10 times) larger than emulsions elute with the void peak at the preferable conditions used for emulsion characterization [1]. This prevents a comprehensive size characterization of such mixtures.

In this study a Sedimentation FFF method was developed that separates blood components and PFC in a single run. Effect of blood and blood-free drug mixture on the size distribution of PFC emulsions was investigated.

References
Fractionation of Industrial Starch Polysaccharides by Field-Flow Fractionation

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Starches are important macromolecules with many industrial applications, particularly in the food and pharmaceutical industries. Their uses range from gelling agents to drug transport and even energy storage. Properties of starches vary based on the plant or bacterial source and are connected to the amylose and amylopectin content. Amylose is a linear polysaccharide with α-1,4 glycoside bonds and has a molecular weight around $10^5$ Daltons. Amylopectin is a highly branched polysaccharide with α-1,6 glycoside bonds along a α-1,4 glycoside bond chain backbone and has a molecular weight around $10^7$ Daltons [1].

Characterization of starch has been performed using size-exclusion chromatography (SEC). The use of SEC for polysaccharides is limited by low exclusion limits and shear degradation of the ultrahigh molecular weight components. Field-flow fractionation (FFF) is an alternative to SEC. In 1994, Lou et al. reported the use of thermal FFF (ThFFF) to perform the separation of similar polysaccharides (i.e. pullulan) with varying molecular weight and of corn starch with a varying amylose and amylopectin content [2]. ThFFF uses a temperature gradient as a perpendicular force to promote the fractionation of analytes based on the ratio of thermal diffusion to normal diffusion. Retention of the analytes is therefore controlled by a temperature gradient across the hot and cold walls and the molecular weight of the analyte. Essentially, larger molecular weight species will be retained longer in the thermal channel than lower molecular weight species, in the normal mode of separation. In the past decade, flow FFF has been in the spotlight with respect to polysaccharides analyses [3-5] in an aqueous system. Separation of polysaccharides of starches by flow FFF is based on molecular diffusion and size of the macromolecule.

This work will showcase the use of FFF for the analysis of various industrial starches. Investigations of various sample preparation techniques (including the use of an ionic liquid) will also be discussed.

References:
Indoor airborne particles have been collected using different sampling methods including two newly designed equipments.

One is consisted of a series of glass cylinders containing water through which air is forced to pass by a vacuum pump. The airborne particles are trapped in water while air passes through the cylinders.

Another is consisted of a pair of oppositely charged parallel plates, one of which is immersed in water. No pump is used. The airborne particles are collected in the water.

The collected airborne particles were analyzed by asymmetrical flow FFF (AsFIFFF) and Sedimentation FFF (SdFFF) for size determination. The results from AsFIFFF and SdFFF were compared with those from photon-correlation spectroscopy (PCS). The airborne particles were also analyzed by atomic absorption spectroscopy (AAS) and ICP-MS for determination of elemental composition. The airborne particles were filtered through a 0.8 μm filter to remove large particles. Results may suggest a sampling and total analysis method of airborne particles.
Characterization of Fly Ash using Field-Flow Fractionation, Dynamic and Static Light Scattering

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Thermal power plants exhaust large quantity of combusted waste in the forms of bottom ash, fly ash or slag. The generation of fly ash by thermal power plants has long caused on environmental problems with technological and economic effects in the world. Fly ash’s potentially hazardous nature is primarily due to the volatile toxic metals that it contains.

In this study, the fly ash exhausted from thermal power plants has been analyzed using AAS and ICP-MS for elemental analysis and field-flow fractionation (FFF) for separation and size-analysis. The applicability of field-flow fractionation (FFF) was investigated. Among FFF sub-techniques, asymmetrical flow FFF (AsFlFFF) and Sedimentation FFF (SdFFF) were chosen because they are applicable to separation and characterization of aqueous suspensions of nano to micron-sized particles. The results obtained from AsFlFFF and SdFFF were compared. The results from AsFlFFF and SdFFF were then compared with results obtained by Dynamic light scattering (DLS) and Multi angle light scattering (MALS).
Advances in Programmed Field Decay Thermal Field Flow Fractionation of Polymers: Direct and inverse calibration methods.

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New procedures [1,2] for determining the calibration function able to relate retention and operative parameters to molecular weight of the species in Thermal Field Flow (ThFFF) under Thermal Field Programming (TFP) conditions are discussed and compared. These procedures rely on the average values of retention parameters under TFP and pertinent a numerical procedures able to relate retention to the temperature variations that occur during TFP.

The first procedure [2] is based on determination, for each retention time position, of the average lambda retention value typical of TFP ThFFF. This parameter is then used to obtain the calibration plot (i.e. the molecular weight of the species as a function of the retention time position) by using the programming function and the calibration plot under varying $T_c$ values. This requires the knowledge of the calibration plots in terms of lambda vs the molecular weight as well as the cold wall temperature and the thermal gradient.

In the second procedure [1] the calibration parameters are obtained by fitting the retention and operative parameters that hold true at the beginning of the TFP. The procedure is closely related to the one previously developed to calibrate the retention time axis under TFP ThFFF and, together, they constitute a full calibration procedure. Experimental validation was performed with reference to Polystyrene (PS) -decalin and PS-tetrahydrofurane (THF) systems.

The presented approach can be conceptually extended to other FFF subtechniques, enable thus to reach new applications of the concept of absolute and/or universal calibration capabilities of the FFF techniques under generalized Field Programming operation, with respect to the pertinent species property to which the specific FFF subtechnique is related.

Effects of Surface Modification on the Retention of Gold Nanoparticles in Asymmetric Flow Field-Flow Fractionation

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Nanotechnology platforms offer many advantages for drug delivery and medical diagnostics. Currently, several concepts utilizing gold nanoparticles are in preclinical development and clinical trials; examples include diagnostic agents [1], thermal ablation therapies employing gold nanoshells[2], and gold particles for drug and gene delivery [3,4]. Thorough physicochemical characterization of these therapies is critical for regulatory approval and commercialization, and for particles with polyethelene glycol (PEG) surface coatings this includes measurement of the extent of the PEG coatings and quantification of the amount of PEG attached to each particle. This information is not readily accessible from a conventional Transmission Electron Microscopy (TEM) or Dynamic Light Scattering (DLS) measurement of particle size.

Here we quantitatively measure thiolated PEG coatings on 30 nm gold nanoparticles by PEG-displacement with dithiolthreitol (DTT) using chromatography and colorimetric assays. We also measured the effect of the surface modification on the retention behavior in Asymmetric Flow Field-Flow Fractionation (AFFF) as detected by Multi-angle Laser Light Scattering (MALLS), RI, UV, and in-line dynamic light scattering (DLS). This measurement is then used to examine the effects of the PEG coating on AFFF particle retention.

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References
Study of Calsequestrin Aggregation by Flow Field-flow Fractionation with Light Scattering Detection

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Flow field-flow fractionation with multi-angle light scattering detection indicates that calsequestrin forms even-numbered aggregates, supporting the view that this calcium binding protein aggregates through the interaction of dimers. Contrary to previous reports based on size exclusion chromatography FIFFF further indicates that the dimer is the stable species, with very little monomer present under the conditions analyzed in this study. Increasing the concentration of potassium ion (100-700 mM) causes the dimer to be the increasingly dominant species over monomer, tetramer, and other aggregate species. Increasing the concentration of calcium ion (3-10 mM) causes increased aggregation of dimers into higher order species. Finally, addition of small amounts of the anthracycline analog trifluoperazine (0.10 – 0.50 mM), which is known to disrupt calsequestrin function, induces severe aggregation.

Characterization of Lipoproteins by Flow Field-Flow Fractionation

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Cholesterol is transported in the body by lipoprotein carriers. The serum levels of low-density (LDL) and high-density lipoproteins (HDL) are important parameters in health risk analysis, with LDL generally regarded as 'bad' and HDL as 'good' cholesterol. However, many clinical studies have shown that the situation is much more complex than this. Various subfractions of LDL and HDL can be discerned, with different functions in the body and different cardiovascular risk aspects. Moreover, the important role of very-low density (VLDL) lipoproteins is now recognised.

It has already been shown in previous studies that LDL and HDL fractions can be separated by flow FFF, since they have strongly different average particle sizes (of approx. 20 and 10 nm, respectively) [1-3]. In the project presented here the suitability of AF4 for the characterization of cholesterol lipoproteins has been studied in more detail. Different linear and exponential-decay cross-flow programs were tested in order to optimise the separation of different lipoprotein fractions. For detection a multi-angle light-scattering detector in combination with a UV detector was used. The usefulness of staining the lipoproteins before the separation (with a fat-soluble dye as Sudan Black) was investigated.

A preliminary study was carried out to find a possible relation between lipoprotein patterns and certain clinical syndromes, using volunteer and patient samples provided by the university hospital.

Mitochondrial Proteome Analysis by Frit Inlet Asymmetrical Flow Field Flow Fractionation and Nanoflow LC-ESI-MS-MS

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Mitochondria play an important role in energy production, ion homeostasis, fatty acid oxidation, intracellular signaling, and the regulation of oxidative stress in cell. In relation to human health, mitochondrial deficiencies or dysfunction are thought to be related with neurodegenerative diseases such as Parkinson’s disease, Alzheimer’s disease, Huntington’s disease, and even with the aging process in general. For the study of those diseases, a profound understanding of mitochondrial proteins in terms of their locations, function, and their biological pathways is needed. However, there is no proper method to isolate mitochondria from other subcellular species of cell except density gradient centrifugation.

In this study, we utilized flow FFF for the fractionation of mitochondria extracted from rat liver using centrifugation procedure and for the size characterization as well. For the examination of morphologic differences of mitochondria collected during FIFFF run, confocal microscopy was utilized. Analysis of mitochondrial proteome was carried out first by one-dimensional gel electrophoresis to verify the heterogeneous protein species according to their retention time in FIFFF run. Moreover, mitochondrial proteome of each fraction were lysed and digested to peptide mixtures for the separation and characterization of proteins by nanoflow liquid chromatography-electrospray ionization-tandem mass spectrometry (NanoLC-ESI-MS-MS).
A New Approach for High Speed Non-Gel Based Two Dimensional Proteome Fractionation: Development of Isoelectric Focusing-Multichannel Asymmetrical Flow Field-Flow Fractionation

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Proteome analysis requires a comprehensive and systematic approach that may include high performance separation methods, mass spectrometric analysis or bioinformatics. Among the analytical methods for proteomics research, mass spectrometry has become a powerful tool which identifies mass and sequence of peptides which results in the identification of protein with the help of database search. However, there is always a limitation that mass spectrometry can not resolve all the peptides simultaneously, therefore a proper separation of proteins/peptides must be applied prior to mass spectrometric analysis. For a long period of time, 2D-PAGE has been the representative technology in proteomics due to its high resolution. However, it takes a long time in separation and the difficulties in retrieving proteins from gel exist. In our earlier study, a rapid, non-gel based, on-line two dimensional separation method was developed for proteome separation in which protein fractionation was carried out by first exploiting the differences in their respective isoelectric points (pI) in a Teflon capillary using capillary isoelectric focusing (CIEF), followed by a molecular weight (Mw)-based separation in a hollow fiber by flow field-flow fractionation (HF FFFF). Since IEF was carried out in a capillary in CIEF-HF FFFF, there was a limitation of high throughput analysis. In this study, a multichannel asymmetrical flow FFF (AFIFFF) channel was developed to carry out 2D separation of proteome by the difference of pI’s using IEF and of MW’s using AFIFFF. At the beginning end of the multichannel AFIFFF system, IEF can be carried out first in the lateral direction followed by the focusing/relaxation. Then the sample bands are separated by their MW’s along the multichannel AFIFFF having 6 lanes simultaneously. In the initial evaluation, several protein standards were used for the performance test of IEF-FFF system and later, a real proteome sample was tested. The identification of proteins was carried out by nanoflow-LC-ESI-MS-MS after a tryptic digestion of each collected fraction.
Multidimensional Proteome Analysis of C.glutamicum Lysate Using HF-FIFFF and 2D-Nanoflow LC-ESI-MS-MS

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C.glutamicum is widely used bacteria in bioindustry, due to its remarkable ability to secrete a considerable amount of glutamic acid in specific condition. About 1.5 million tons of glutamic acid is produced in a year from C.glutamicum. Additionally, It is regarded as generally safe industrial process to produce glutamic acid using this organism. Therefore it has been the subject of genomics, metabolomics and biochemistry for a well-defined fermentation process. Identification and characterization of proteome will be a key to research about mechanism in a C.glutamicum cell.

HF-FIFFF (Hollow Fiber Flow Field Flow Fractionation) has been utilized for the fractionation of C.glutamicum in this study. Separation of proteome from C.glutamicum is carried out in hollow fiber made of polysulfoneIts, and the fractions collected during HF-FIFFF run were enzymatically digested. The resulting peptide mixtures of each fraction were analyzed by on-line two dimensional nanoflow liquid chromatography (strong cation-exchange chromatography followed by reversed-phase liquid chromatography) and tandem mass spectrometry. A home made dual trap was utilized by sequentially packing C18 reversed-phase (RP) particles and SCX resin in a silica capillary tubing (1.5 cm x 200 µm I.D. for SCX, 0.7 cm x 200 µm for RP) ended with a home-made frit and is connected to a nanoflow column having a pulled tip treated with an end frit. The high speed separation of HF-FIFFF may become an advantage over the gel-based technique for the fractionation and collection of proteome mixture. With the pre-fractionation of C.glutamicum proteome using HF-FIFFF, 118 proteins were additionally identified from the number of identified proteins using 2D-nanoflow LC-ESI-MS-MS alone.

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The aim of the present study was to isolate neural stem cells from a complex tissue: the mouse olfactory epithelium. Recently [1], we demonstrated, that by using "Hyperlayer" elution mode, fraction collection and cell characterization methods, that Sedimentation field flow fractionation (SdFFF) could be a useful cell sorter to isolate an immature neural cell fraction from the avian olfactory epithelium. Cells were able to generate neurosphere-like structures which were composed of cell having many features of stem cells: undifferentiated, self-renewable and multipotential. Such a population might be used as a model to improve our understanding of the mechanisms of olfactory neurogenesis in adults. Nevertheless, the avian model has many disadvantages such as the absence of specific markers to perfectly characterize the eluted cell population. For this reason, we chose to use our experience in order to develop a mammalian model. It provides many advantages: (i) more similar to humans; (ii) the availability of specific markers for cell characterization. To improve basal cells (stem cells) collection and separation from other cell types (olfactory neurons, sustentacular cells), mice were treated (IP injection) with 2,6-dichlorobenzonitryl which specifically deleted olfactory sensory neurons and supporting cells but spared the basal cells. Our results showed for the first time, that SdFFF was able, in comparison to a control population, to monitor the kinetics of epithelium destruction (maximal 72h after injection). After fraction collection, preliminary results demonstrated the possibility to isolate cellular fractions which also led to neurosphere-like structures: a tool to study the properties of neural regeneration and differentiation of SCO-spondin. This is a multidomain glycoprotein belonging to the family of thrombospondin. This protein and deduced peptides have been shown to appear early during embryonic life and to be able to act on nerve cells differentiation. Preliminary results suggested two different activities for the peptide: a) effect on the early differentiation of ES cells: this effect was mainly observed on the kinetics of the differentiation rather than induction itself b) effect on the potentiation of the neuronal progenitors with a proliferation of the cells suggesting an increase of the response to the presence of the growth factor FGF2. This last role of the SCO peptide in the development of neural precursors number seems very promising to improve in vitro differentiation but also potentially to recruit and amplify in vivo the low percentage of progenitor stem cells in an adult animal. The isolation of basal cells from the olfactory epithelium by SdFFF provides a new tool to analyze the effect of SCO-spondin on regulating differentiation and proliferation from adult neural stem cells. These results will help to confirm the interest of these compounds in the treatment of a wide range of Central Nervous System disorders including neurogenerative diseases and axonal degeneration particularly by promoting neuroprotection and neuroregeneration.

Relationship between Durum Wheat Dough Strength Properties and Protein Size Distribution as Determined by Flow Field-Flow Fractionation.

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Size distribution of gluten proteins is purported to be related to dough strength properties. Flow field-flow fractionation (FFF) has been effectively applied to the study of gluten proteins in common wheat (Triticum aestivum). Very little, however, has been reported on the use of this technique with durum wheat (T. turgidum var. durum). Common wheat (hexaploid) and durum (tetraploid) appear to have different strength mechanisms with low molecular weight (LMW) glutenins being more important contributors in durum wheat whereas high molecular weight (HMW) glutenins are the major contributors to dough strength in common wheat. In addition, there is a lack of consensus regarding the role of high molecular weight glutenin subunits (HMW-GS) in relation to durum strength. In this study, samples of durum semolina were reduced to produce flour by repeated passage through sizing rolls to improve protein extractability. They were then extracted using a sequential extraction procedure with 0.05M acetic acid without, then with sonication to remove mainly monomeric (readily extractable) and predominantly polymeric (sonicated) proteins respectively. Five HMW-GS patterns were identified within the sample set: 6+8; 7+8; 14+15; 20 and 2*, 20. Samples were further categorized by LMW-GS into two classes: LMW-1 (weak) and LMW-2 (strong). Size distribution of extracted protein was measured using symmetrical flow FFF. Combustion nitrogen analysis (CNA) was used to measure the amount of protein which remained unextracted in the residue. Dough strength was determined by Brabender Extensograph, gluten index (GI) and unextractable polymeric protein content (%UPP). Chemical composition as indicated by glutenin/gliadin (Glu/Gli) and HMW/LMW-GS ratios was also assessed and compared with size distribution data. The readily extractable protein was negatively correlated (p<0.05) with strength parameters whereas the protein requiring sonication for extraction was highly positively correlated (p<0.001). Dough ‘extensibility’ (L) was not influenced by protein size distribution. No significant correlation was found between protein remaining in the residue and any of the strength indicators. The fractions of the readily extractable protein were generally negatively correlated with %UPP and Glu/Gli, with the exception of the fraction containing the very large polymeric material (>37.5nm) which showed a positive relationship with Glu/Gli. Only the material extractable with sonication showed correlation with HMW/LMW-GS. The negative correlation suggested that unlike common wheat, where larger polymeric material is associated with increased dough strength, in durum wheat an increasing relative proportion of HMW-GS corresponds to a decrease in dough strength.
Affinity-based Protein Pre-fractionation by Flow-Field Flow Fractionation

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We are developing a method, called Multiplexed Affinity-based Protein Pre-fractionation (MAPP), to simultaneously extract protein subsets with antibody-bound microspheres. The antibodies have high affinity to the bait proteins, and microspheres with different sizes are conjugated with different antibodies to target various bait proteins. Via extraction of the bait protein, we also expect to fish out proteins tightly associated with the bait proteins. Then separate the microspheres by flow-field flow fractionation before eluting proteins from the collected microsphere fractions. MAPP eliminates sample variations introduced from sequential isolation processes and enhances purity of the extracted proteins from streamline separation. Therefore, this method can help to identify proteins that work as a complex in cells and understand the expression and composition relations among different complexes.
Study of the Growth Rate of *Saccharomyces cerevisiae* Strains using Wheat Starch Granules as Support for Yeast Immobilization Monitoring by Sedimentation/Steric Field-Flow Fractionation

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The efficiency and the effectiveness of wheat starch granules as a support for the immobilization of an alcohol resistant psychrophilic yeast *Saccharomyces cerevisiae* AXAZ-1 was studied. The growth rate of these cells in the presence or the absence of the support in the culture medium was investigated by the technique of Sedimentation/Steric Field-Flow Fractionation (Sd/StFFF). An abrupt increase of biomass productivity in less required time was observed in the case of the presence of wheat starch granules in the culture medium. The results indicate that wheat starch granules might be a good medium for yeast cell culture and bioreactor formation.

**Key words:** Yeast immobilization, Yeast cells proliferation, Biocatalysts, Wheat starch, Field Flow Fractionation

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Enrichment of Adipose-Derived Stem Cells using Dielectrophoretic Field-Flow Fractionation

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The relevance of stem cells to tissue regeneration and tumor progression is becoming evermore apparent. Elucidation of the fundamental biology and eventual clinical application of multipotent cell types will require the isolation and characterization of relatively rare cell subpopulations from complex mixtures of cells and biomolecules. Conventional cell separation methods exploit size or density differences to enable the discrimination of different cell types. More refined methods employ cell-specific molecular surface markers that are labeled to facilitate separation by flow cytometry (fluorescence-activated cell-sorting or FACS) or magnetic columns (magnetic cell sorting or MACS). While useful, current methods are limited in that they require a priori characterization of the physical and biochemical properties of the cell subpopulations of interest. We are investigating the use of dielectrophoretic field-flow fractionation (DEP-FFF) to prepare multipotent cell populations from autologous tissue sources.

DEP-FFF is a field-flow fractionation technique that exploits intrinsic differences not only in cell size and density, but also membrane morphology and integrity as well as intracellular organization to enable discrimination of cell subpopulations and diseased or abnormal cell types. The method has been applied to the separation of cancer cells from normal cells, blood cell subpopulations, viable from apoptotic cells and bone marrow derived CD34+ hematopoietic stem cells from tumor cells. We have recently studied the suitability of the method for isolating putative stem cells from adipose tissue. Excised subcutaneous adipose tissue or lipoaspirate was enzymatically digested and the resulting cell mixture subjected to DEP-FFF processing. Specifically, flow cytometric methods were used to follow the elution of fluorescently immunolabeled populations of cells within the tissue digest during the DEP-FFF separations. Putative stem cells were pre-labeled with antibodies against either NG2 or nestin, pan-leukocytes with anti-CD45 antibodies and erythrocytes with anti-glycophorin A antibodies. Using this method, we observed separation of damaged cells, cellular debris and erythrocytes from the white cell populations, and NG2 or nestin-positive cells were enriched by up to 14-fold. We are now working to improve the performance of the method for harvesting of stem cells and other rare cell populations from complex cell mixtures.
Bovine Serum Albumin Aggregation Studied by Asymmetrical Flow Field Flow Fractionation Connected to UV, Dual Angle Light Scattering, RI and Viscosity Detectors.

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Depending on the native protein structures, aggregation or unfolding is induced by several kinds of treatments, such as acidification, addition of salt, treatment with hydrolytic enzymes or chemical reagents, and heating.

In the present work, we studied the heat-induced aggregation of bovine serum albumin (BSA) using asymmetrical flow field flow fractionation connected to UV, dual angle light scattering, RI and viscosity detectors. After heating, aggregates were formed and their sizes were affected by the aggregation temperature, time of heating, protein concentration and the presence of NaCl or SDS. Temperatures from 50 to 80 °C were used for the heating of samples, the protein concentrations were varied between 0 and 10 mg/ml for a time duration of 0-480 minutes in the presence of 0-2 M NaCl or 0.05% SDS. The hydrodynamic diameter of the native monomer in 8.5 mM phosphate buffer at pH 7.4 with 150 mM NaCl at ambient temperature was ~7 nm, whereas those of the aggregated particles, heated at 80 °C were 20-40 nm depending on the protein concentration and heating time. The critical aggregation temperature of BSA (1 mg/ml) in the presence of 150 mM NaCl was ~63 °C. Beyond the critical aggregation temperature, the higher the temperatures the larger were the particle sizes formed. When the concentration of NaCl was increased to 1 or 2 M, the critical aggregation temperature was ~70 °C. The critical concentration to form aggregates at 80 °C was 0.1 mg/ml, and the time needed was about 2 minutes for 1 mg/ml. Beyond the critical concentration, the higher the protein concentration the larger were the particle sizes formed. Treatment of 1 mg/ml BSA solution at 80 °C with SDS below or above CMC did not produce any aggregates. A dual angle light scattering detector was used to estimate the molar masses and sizes of the protein aggregates. From the relation between the apparent diffusion coefficients and the molar masses of the aggregates, as well as from the ratio of the root mean square, and the hydrodynamic radii, the possible conformational structures of the aggregates were determined.
Wall-Antibody Immobilization to Hybridize Gravitational Field-Flow Fractionation for Antigen-Specific Particle Sorting

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The need of simple and highly selective biocompatible methods for rapid purification of analytes such as nucleic acids, proteins or whole cells from complex biological matrices is increasing, particularly in the forefront of biotechnology and analytical proteomics. Immunological and DNA hybridization methods are currently available for the fast and sensitive detection of these bioanalytes [1].

Among FFF techniques, gravitational FFF (GrFFF) uses a low-cost channel suitable for a disposable use, and simple and economic instrumentations. Recently, we proposed an innovative flow-assisted immunoassay format based on GrFFF for the quantification of pathogenic microorganisms present in food or other biological samples [2]. Biospecific (e.g. antigen-antibody or ligand-receptor) reactions might be also exploited to improve selectivity of GrFFF-based cell sorting techniques through the application of a hybrid GrFFF mechanism.

In this work, we propose the immobilization of a specific antibody on the accumulation wall of a GrFFF channel to improve selectivity of the technique. With this approach, the retention behavior of a specific analyte is selectively modified by the specific interaction with the antibody immobilized on the channel wall. The separation is achieved by the balance between the affinity constant of the analyte with the immobilized antibody and the gravity-assisted flow transport into the channel. Parameters as channel materials and thickness, and mobile phase composition have to be optimized to control this balance with respect to the morphological characteristic of the analyte (protein or cells).

Micrometer-sized polystyrene beads covalently coated by a monolayer of horseradish peroxidase (HRP) have been used as a model sample. A specific anti-HRP antibody was immobilized on the accumulation wall in correspondence of the channel inlet. The HRP activity of the eluted beads was detected by chemiluminescence (CL). The CL signal was detected both by a flow-through luminometer and by imaging. Imaging was accomplished by placing the GrFFF channel in a dark box and by acquiring, through a highly-sensitive, back-illuminated, double Peltier-cooled CCD camera, the CL emission at fixed time intervals during the elution [3]. The possibility to perform in-channel visualization of the sample band evolution gave information on the fractionation process, and on the onset of the sample-wall interactions required to develop the method. The specific sample/antigen-antibody/wall interactions occurring during relaxation actually caused shape and intensity modifications of the characteristic retention peak. This finding demonstrated the effective onset of selective antigen-antibody interactions. The hybrid method combines the advantages of a simple and soft separation technique with the selectivity of a biospecific interaction. In case of cell sorting, it could also make it possible selective cell identification in multiplex format.

Effect of Temperature on Separation and characterization of Monoclonal Antibody using Asymmetrical Flow Field-Flow Fractionation.

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Stressed and non-stressed monoclonal antibody samples were analyzed at a temperature range of 20-80 °C using the \textit{Mid Temperature AF2000 system (AF2000 MT)}. Three important effects were observed. Firstly, increasing the system temperature to 40 °C and above resulted in narrower monomer peaks with reduced dilution effect. Secondly, the thermal stability of protein can be directly monitored during the FFF run. Thirdly, the thermal unfolded intermediate species can be separated and characterized.

This study demonstrates that the use of MT AF2000 for protein separation can result in an enhanced resolution. It provides a simple and quick method to evaluate the thermal stability of protein. This system can also provide the size distribution of proteins at temperatures close to that of human body which is not possible using the currently available FFF systems.
A MEMS-based magnetic cell fractionation and detection device: design, fabrication and testing

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The need to rapidly detect pathogenic microorganisms in the field demands a small, mobile, high-speed device, for which Micro-Electrical Mechanical Systems (MEMS) technology offers the best prospect. The design and fabrication of a biochip for a portable diagnostic device - based on an existing macro-scale magnetic cell multi-fractionator - will be presented. The microfluidic channel network and magnetic components were designed so that cells can be introduced, fractionated and trapped on the chip for further analysis. Magnetically-tagged cells are fractionated based on their magnetophoretic mobility (MM). Suspended cells pumped at low Reynolds number into a flow channel positioned in a magnetic field with constant energy density gradient ($\nabla B^2$), displace orthogonal to the flow according to their MM. The displaced cells are separated into six outlets in a manner specific to a given cell type, and are magnetically captured downstream by a 4-magnet array of increasing (katodynamic) gradient. Immunomagnetically-tagged Jurkat and KG-1a cells, as well as custom magnetic microspheres, were used as the model particles. Particle trajectories were simulated numerically (Runge-Kutta method) using MM data - which was experimentally determined by Cell Tracking Velocimetry (CTV) - to predict the separation outcome. A constant magnetic gradient was obtained by a set of discrete ferromagnetic elements (pole pieces) imbedded on both walls of the magnetic separation channel, magnetized by an external permanent magnet assembly. The biochip was fabricated using two quartz wafers as substrates and multilayer SU-8 photo-resists as the structural elements. Adhesion of SU-8 to quartz was improved by patterning a 10 µm SU-8 layer before the thick layer is applied on the wafer. SU-8 (120 µm thick) electroplating masks were fabricated on one side of both wafers. The SU-8 (250 µm thick) channel network was patterned on the other side of one wafer and the two wafers were bonded using SU-8 adhesive bonding to form enclosed channels with discrete pole pieces plated on opposing walls.
Characterization of Functionalized Styrene-Butadiene Rubber by Organic Solvent Flow Field-Flow Fractionation/Light Scattering

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Flow field-flow fractionation (FIFFF) using an organic solvent as mobile phase has been effectively utilized for the separation and characterization of functionalized styrene-butadiene rubbers (SBR) that are polymerized and followed by coupling reaction in solution. Separation of broad molecular weight SBR was accomplished by an asymmetrical FIFFF channel in THF under field programming and the molecular weight distribution (MWD) of the SBR sample was determined by on-line measurement of light scattering. In this study, FIFFF has been utilized to characterize high-MW functionalized SBR from the low-MW non-functionalized molecules which were used for coupling reaction to produce high-MW functionalized SBRs, and to determine the coupling number of the functionalized SBRs depending on the type of the coupling reagents. The resulting MWD of the SBR samples prepared by the different coupling reagents (SnCl$_4$ and a polydimethylsiloxane compound) were compared. It is shown that AFIFFF coupled on-line with light scattering (LS), AFIFFF/LS, can be powerfully utilized for the monitoring the difference of MWDs and the coupling number according to the preparations.
Laboratory Designed Development of Hollow Fiber Field Flow Fractionation (HFFFF) for Macromolecules Analyses, The Example of High And Polydispersed Molecular Mass: Blue Dextran.

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One of the specificity of research development in field flow fractionation is its absolute low cost if instrumentation tasks are accepted. Recently; Kok, Moon and Reschiglian works on HF FFF demonstrated that this approach may appear clever if one wants to enter FFF directed by hydrodynamic fields. It is shown in this report that combining bibliographic instrumentations descriptions led to the development of specific devices specially designed to encompass hollow fibers commercially available. In particular the sealing of the hollow fiber to connection tubings or field and flow generators has driven the designers to customize some specific features. The HFFFF system therefore designed allows establishing relative high pressure leading to large field / flow balances at high flows.

Systematic works; using a very polydispersed, high molecular mass, blue dextran; are therefore described in order to valid the instrumental set up.
In particular it appears that the so called “focalisation” or relaxation process must be studied with care in terms of injection volume and technical “switching procedures”.

Compared to the two sedimentation methods (gravitational and multigravitationnal) already conceptualized in the laboratory the cinematic of the development of a usable device in HFFF appears very interesting. Such device simple to set up, rapidly operative and versatile can be at roots for FFF development in laboratories with limited instrumentation skills or willing.

HFFFF Amersham UFP E 5A 160X1 (L*id)
H2O blue dextran (75 uL)

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Dextran, flow rate 0.2 ml/min, radial field 73.2%, focusing time 4 min, injection with focusing mode.
Numerical Simulations of Transport Processes in Electrical Field-Flow Fractionation System

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The effective field in the presence of the electrical double layer in electrical Field-Flow Fractionation (ElFFF) is a small fraction of the overall field and a strong function of a number of operational parameters such as: the materials and electrochemistry at the electrode interface, carrier flow velocity, and the ionic strength and pH of the carrier solution. The unavailability of accurate models to predict the effective field for changes in operational parameters has resulted in inadequate retention models for ElFFF. This paper presents an improvement over the previous communications and includes a 2-dimensional multiphysics numerical model for particle transport in ElFFF.

Several groups have attempted to devise transport models for ElFFF and cyclical ElFFF, but these models either use empirical values for the effective field or do not take in to account the electrochemical effects occurring at the electrode-carrier interface to estimate the mass transport and retention behavior [1,2,3]. Use of electrical circuit analogue is an effective way to model retention characteristics incase of ElFFF [4,5,6]. A thorough analysis of such an empirical model based on the electrical circuit parameters presented a unique way to calculate effective field and establish current across the ElFFF electrodes as more accurate operational barometer of the retention compared to voltage in a communication from our group [4]. In this work, current is used as the driving force for the particle migration under the influence of electrical field. An expression for parabolic velocity profile in the channel is used to define the convective part of the particle mass transport. The 2-dimensional model presented in this paper uses COMSOL multiphysics software (COMSOL, MA). Plate height and retention time are calculated as a function of field, carrier ionic strength and flowrate, and particle charge and size.

Fullerene $C_{60}$ nanoparticles are considered for a variety of biological applications such as imaging probes, antioxidants and drug delivery. Size characterization of nanoparticles using methods such as light scattering often gives values in the order of tens or hundreds of nanometers. Interpretation of the results becomes even more complicated when the medium contains ions that result in aggregation of nanoparticles.

In this study we used Asymmetrical flow field-flow fractionation (AsFlFFF) to determine the size distribution of soluble $C_{60}$ ($C_{60}$-OH) nanoparticles in aqueous solutions of different pH and ionic strength. A 100 times increase in the ionic strength, resulted in about 60% increase in the size of the nanoparticles. However changing the pH from 6 to 10 did not result in a significant difference in size. The obtained particle size was verified using Atomic force microscopy.
Effect of Channel Angle On Retention of Polystyrene Micro Beads in Gravitational Field-Flow Fractionation (GrFFF)

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Gravitational Field-Flow Fractionation (GrFFF) is a member of FFF family that is useful for quick-and-easy separation and characterization of various types of particles ranging in diameter from a few to about $10^2$ microns. Compared to other members of FFF, GrFFF is relatively simple to setup and easy to operate. One of limitations of GrFFF is that the external field available in GrFFF is the earth’s gravity, which is constant (not tunable), and relatively weak, limiting applicable size range of GrFFF higher than a few microns. The external field strength can be varied by changing the channel angle.1

In this study, the GrFFF channel angle was varied from 0° to 60° to study the effect of the channel angle to the retention of micron-sized polymeric latex beads. The channel thickness was also varied. The retention ratio ($R$), resolution ($R_s$) and the plate height ($H$) measured at various channel angles and at different channel thicknesses were compared. The results were also compared with theory.

References

Theory for Nanoparticle Retention Time in Quadrupole Magnetic Field-Flow Fractionation

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Quadrupole magnetic field-flow fractionation (MgFFF) is an analytical and characterization technique for particulate magnetic materials [1-4]. An important application is the characterization of the superparamagnetic nanoparticulate reagents used for labeling biological cells for their enrichment or isolation. These composite nanoparticles are typically composed of nanosized magnetite or maghemite cores coated with a biocompatible material such as dextran and then with antibodies to specific cell surface antigens. When a cell that is labeled with these nanomaterials is placed in a magnetic field, the magnetic spins become aligned with the field and the cell may be considered magnetized. It may then be driven in the direction of an applied gradient in magnetic field strength. The separation of labeled cells from unlabeled cells may thereby be realized in high gradient magnetic separators (HGMS) or in the quadrupole magnetic flow separator (QMS).

Quadrupole magnetic field-flow fractionation uses a thin helical channel mounted in a radially symmetric field gradient. Magnetization of the nanoparticulate sample is induced by the applied magnetic field and it follows that the particle-field interaction parameter is a function of the applied field strength. The fluid velocity profile in the thin helical channel deviates from the parabolic profile found in a parallel plate channel. The influence of the various experimental conditions and instrumental parameters on particle retention time will be discussed, and the relevant equations presented.

References
Evaluating Flow-Through Photon Correlation Spectroscopy for the Measurement of Diffusion Coefficients for Polystyrene and Proteins

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Flow-through photon correlation spectroscopy (PCS) was introduced several years ago as a means for measuring the diffusion coefficients (D) of macromolecules and particles as they eluted from a separation column or channel. Hydrodynamic radii can be calculated from the measured D values by using the Stokes-Einstein equation. When these values are combined with the retention time measured by field-flow fractionation, thermal diffusion coefficients and shape information can also be inferred.

Photon correlation spectroscopy is based on the time dependent fluctuation of the intensity of light scattered by the analyte. The scattering intensity fluctuations arise from the Brownian (random) motion of the analyte in solution. The time dependence of the scattering intensity can be used to derive D through an autocorrelation function. The batch mode setting is the preferred way to measure D. Advantages include the ability to precisely control the analyte concentration and analysis times to achieve strong analyte signals. However, batch mode measurements require a substantial amount of sample and can be tedious, particularly, when examining fractions collected after a separation stage. A recently offered alternative to batch mode PCS measurements is flow-through PCS measurements. An advantage to flow-through PCS is that it can be connected on-line with a size exclusion chromatography (SEC) column or a field-flow fractionation channel providing less sample handling and reduced analysis time. Possible complications arising from flow-through PCS measurements include additional axial translational motion of the analyte as a result of the flow rate used to accomplish separation, and weak scattering signals of synthetic polymers in organic solvents.

Despite the accepted use of flow-through PCS, there has been little to no detailed investigation of the experimental conditions required for accurate measurement of D. The goal of this study is to evaluate the accuracy of D values obtained as a function of flowrate and sample concentration and molecular weight. The range of separation conditions span those typically used in size exclusion chromatography and field-flow fractionation. The analytes are polystyrene polymers prepared in organic solvents and proteins prepared in aqueous buffer solutions. Comparison of batch- and flow-through PCS values suggested that the analyte concentration has the largest impact on D values obtained by the latter. These studies have enabled us to define guidelines for accurate flow-through PCS operation.
Tandem Hollow-Fiber Flow Field-Flow Fractionation

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Re-injection of one or more collected fractions of the eluted sample is widely recognized as a useful “tandem” procedure in separation methods, including field-flow fractionation [1]. It is valuable support for method optimization, since it provides information about the actual separation performance. In case of complex samples, re-injection may also improve resolution, and discover artifacts due to concentration effects or interactions between the sample components. In most cases, however, off-line re-injection is a labor intensive procedure, mostly because the analyte dilution during first run often makes it necessary a re-concentration step of the collected fractions before the second (re-injection) run.

Hollow-fiber flow field-flow fractionation (HF FlFFF) is the micro-volume, tubular-channel variant of FlFFF [2], which has reached a performance comparable to that of standard, flat-channel FlFFF. In this work, to further improve HF FlFFF performance and application, we present a new device and method for on-line tandem HF FlFFF. For tandem HF FlFFF operations, the instrumental setup was properly modified including a four-port, two-way valve positioned downstream the UV detector. The valve is equipped with a sample loop of proper volume. HF FlFFF2 is carried out in four steps. First, the sample is injected in the channel and subjected to relaxation/focusing. Thereafter, elution is started, and the eluted fraction that will undergo to re-injection is collected in the downstream sample loop by operating the two-way valve. After completion of sample elution (first run), the system is set to relaxation/focusing mode, so that the collected fraction is fed back to the HF channel by the reversed focusing flow stream, and focused at the same longitudinal position that was set for the first run. This focusing step reduces the sample plug volume, and it brings the analytes present in the collected fraction back to their original concentration. The second run is eventually performed under the same or different flow conditions with respect to the first run.

The tandem HF FlFFF performance is evaluated by running some model protein samples. Under optimized conditions, the selected sample band is collected and re-eluted with no significant loss in resolution and sample recovery. Sample recovery can be evaluated by comparing the detector signal in the first and second run, even for samples of unknown concentration and composition for which off-channel re-injection would provide biased results. In the case of protein oligomers, tandem HF FlFFF results particularly effective since it allows to collect and re-inject the bands corresponding to each oligomer. When second-run elution of the collected fraction occurs at the same retention time of the first-run elution, that allows to confirm the presence of a stable protein aggregate. On the other hand, for instance, experimental artifacts due to the high sample concentration typically reached during the relaxation-focusing procedure can be excluded. Tandem HF FlFFF may be, therefore, particularly effective in the case of complex protein samples such as blood serum, since it may improve detectability of low abundance components. Overloading effects due to high-abundance components can be reduced in the second run by re-injecting a selected sample fraction. Decrease of analyte dilution at the channel outlet can also be obtained by eluting the collected fraction at retention lower than in the second run. This can improve detectability if further characterization by off-line methods such as mass spectrometry wants to be performed on selected fractions of particular interest.

Exploration of Shear-Induced Diffusion as a Mechanism for Non Specific Crossover in SPLITT Fractionation

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Split-flow thin channel fractionation (SPLITT fractionation) is most commonly implemented for binary fractionation. The samples, however, tend to be broadly dispersed in the selective property. For example, environmental particulate samples may be fractionated into subpopulations of particle size by successive binary fractionation. In the specific application of separation of magnetically labeled biological cells from unlabeled cells by the special SPLITT system known as the quadrupole magnetic flow separator (QMS) the sample may be truly binary. It is also often of critical importance that the unlabeled fraction does not contaminate the labeled fraction or vice versa. The migration of unlabeled cells across the transport zone to be collected along with the labeled cells is known as non specific crossover (NSC) to distinguish this migration from the specific selection of labeled cells. The optimization of throughput for SPLITT fractionation of these truly binary samples requires the transport lamina to be as thin as possible while effectively eliminating NSC.

Shear-induced diffusion [1-4] is explored as a possible mechanism for NSC. Shear-induced diffusion is predicted to result in significant cross-streamline migration of micron-sized particles in sheared suspensions. The effective diffusion coefficient is predicted to be proportional to the local shear rate and the square of particle radius, and to be a function of the local particle volume fraction. The parallel-plate SPLITT fractionator has been modeled to include the influence of shear-induced diffusion on NSC. The influence of particle size, sample particle volume fraction, system dimensions, and inlet and outlet flow rate ratios have been examined.

References

Study of Split-Flow Thin-Channel Fractionation using The Discrete Element Simulation Method

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The discrete element method (DEM) is a computational simulation approach that has been used for study of particulate systems since 1979. It employs the Newton equations of motion to trace particle positions and velocities in a solid-fluid system. In this method, the discrete volume of individual particles is taken into account, and particle collision – either with other particle or with the vessel walls, as well as the effect of particle-fluid interaction can be incorporated into calculations. As a result, the DEM provides a simulation model closer to the real physical system under consideration in comparison with many other simulation approaches.

This presentation will examine the usefulness of the DEM for studying particle-particle and particle-fluid interactions in SPLITT fractionation. The method was tested against the established SPLITT theory and published experimental data for 7, 10 and 15 µm particles at various particle concentrations and channel flow rates. Two illustrative examples will be presented, (1) the extent of particle collision under typical SPLITT run conditions, and (2) the effect of particle momentum on its surrounding fluid, therefore, on particle trajectories.

These examples show that at a typical sample concentration of 0.1%, around 25% of particles may collide with other particles before emerging from the channel. That finding suggests interparticle and/or lubrication forces may play an important role in SPLITT separation. On the other hand, under very dilute particle concentration conditions, the effect of particle-fluid interactions on particle trajectory is quite small. It is almost unnoticeable for 7 and 10 µm particles, and only a small deviation from the theory to be observed for 15 µm particles. Results for larger sized particles, 20 and 30 µm, will also be included and discussed.
In situ visualization of micron-size particles for FFF and SPLITT fractionation

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The hydrodynamic behaviour of micron-size species during the separation process in FFF and SPLITT channels is rather complex and can become unpredictable even in very simple cases. Separations imply on the one hand, interaction of species, transported by a non-uniform flow, with a field force; on the other hand, separations imply hydrodynamic interactions between particles and walls and between particles themselves. Those interactions lead to differential species trajectories which are difficult to follow. In fact, what we observe is the average behaviour of different species during the separation process by determining either, residence times and peak shapes in FFF, or retrieval fractions in binary SPLITT fractionation.

In situ visualization of separations process in FFF and SPLITT has been rarely tempted; there is a very nice example of separation of particles in gravitational FFF using chemiluminescence technique. In reduced-size FFF and SPLITT channels, the study of individual or finite number of particle behaviour may be enough for predicting separation and channel performances. In situ visualization could become thereby useful for optimizing separation flow and field parameters.

In this poster, we show three different kinds of in situ visualizations of micron-size vesicles and particles flowing in a Hele-Shaw channel undergoing an acoustic radiation force. Acoustic focusing is observed by using Phase Contrast Microscopy, Digital Holographic Microscopy and micro-Particle Image Velocimetry, µ-PIV.

Particle visualized by Digital Holographic Microscopy of silica particles in a 234 µm thick channel. Particles are flowing with an axial velocity of 6 cm/s. Left: instantaneous picture of particles focused at the channel centre (middle picture) and at two symmetric positions -90 µm and +90 µm without acoustic force. Some particles are focused at all positions. Right: pictures with acoustic force, node at the channel centre. Particles are focused only at the centre (middle picture) and any focused particle observed at the other two positions.

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Effects of particle-particle hydrodynamic interactions on the mean magnetophoretic mobility of particle suspensions

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Magnetophoretic mobility, MM, of a particle in viscous media is the result of interaction with the applied magnetic field. For low Reynolds number motion in linearly polarizable magnetic materials (paramagnetic and diamagnetic), single particle magnetophoretic mobility is directly proportional to the difference in magnetic susceptibilities of the particle and the viscous fluid, and inversely proportional to the particle friction coefficient. Differences between particle MM have been used for continuous fractionation of magnetically labeled cells¹. The cell MM was a satisfactory predictor of the cell sorting experimental results at low cell number concentration (10⁵/ml) but not at high concentration (10⁶/ml).

The presence of other particles in the fluid medium introduces perturbation to the particle motion due to particle-particle hydrodynamic interactions². The presence of mobile (MM ≠ 0) particles produces a cooperative effect that may lead to the increase of the apparent, mean MM of the particles³. We have calculated the cooperative effect of particle-particle hydrodynamic interactions on the mean particle MM using Green’s function representation of the fluid flow due to the action of a point source (the magnetic particles), in a constant force (isodynamic) magnetic field⁴, for a finite number of particles (43) in an unbounded fluid. The well-mixed conditions were approximated by keeping the point sources at the nodes of a 3D hexagonal lattice. The effect of ratio of mobile to inert (MM = 0) particle number, particle radius and particle number concentration were investigated. The calculated effects were significant at particle volume fractions as low as 0.05%, which may explain experimentally observed concentration effects on the magnetophoretic cell sorting.

References
Improvements in Microscale Thermal-Field Flow Fractionation Instrumentation

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A thorough investigation of the design considerations of thermal field flow fractionation and characterization of a 25 µm thin microscale thermal field flow fractionation system is reported. A 100 to 500 times volume reduction from mesoscale and macroscale systems warrants customized design and operation of the microscale separation system. Operating conditions of the microscale systems differ from mesoscale system [1] distinctly and require careful consideration while characterizing it. Microscale FFF systems require optimum packaging with minimum possible pre-column and post-column volumes including detector volume. A number of design and operational issues need to be considered while optimizing a microscale field-flow fractionation system including: geometry, extra-column tubing, sample injection and operating conditions and improvements in material selection and arrangement. First, analytical models are used to demonstrate the variation in the performance characteristics: plate height and resolution with important design and operational parameters. This paper reports a microscale thermal field-flow fractionation with improved design [2] in comparison to earlier communications [3] without the use of extensive microfabrication techniques [4]. An optimized microscale thermal field flow fractionation (ThFFF) is fabricated with efficient manufacturing and assembly process that can provide high temperature gradient (~ 10⁶ °C/m) and retention. Single particle retention and binary mixture separation are carried out with polystyrene nanoparticle samples in an aqueous carrier to characterize the device. This microscale TFFF has resulted in retention ratios as low as 0.08 with less than 20 minutes of the analysis time.