iSFFF

Nantes 2024

23rd International Symposium on Field- and Flow-based Separations

OUTLINE

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Program at a glance

PROGRAM

MONDAY JUNE 3RD

10h00
REGISTRATION

10h30 - 12h00 - room 150
PRE-SYMPOSIUM WORKSHOP SESSION 1
TUTORIAL ON FFF
Dr. Steven Trainoff

12h00 - 13h30 - R0 space
LUNCH

13h30 - 15h00 - room 150
PRE-SYMPOSIUM WORKSHOP SESSION 2
DATA MANAGEMENT
Dr. Suzanne Boye
Dr. Martin Geisler

15h00 - 15h30 - R0 space
BREAK

15h30 - 17h00 - room 150
PRE-SYMPOSIUM WORKSHOP SESSION 3
HOW TO MAKE OPTIMIZATION, DEAL WITH TROUBLE SHOOTING
Dr. Enrica Alasonati
Dr. Valentin de Carsalade Du Pont

17h30 - 18h00 - room 150
OPENING CEREMONY

18h00 - 19h00 - R0 space
WELCOME PARTY

TUESDAY JUNE 4TH

8h30 - 9h15 - room 150
KEYNOTE LECTURE 1
Pr. Heinrich Haas
QUANTITATIVE SIZE-RESOLVED CHARACTERIZATION OF MRNA NANOPARTICLES BY IN-LINE COUPLING OF AF4 WITH SAXS

9h15 - 10h15 - room 150
SCIENTIFIC SESSION 1
DIAGNOSTIC PART 1
9h15 - 9h30 Guerrini et al.
9h30 - 9h45 Abdulrahman et al.
9h45 - 10h00 Drexel et al.
10h00 - 10h15 Tilli et al.

10h15 - 10h45 - R0 space
BREAK

10h45 - 12h15 - room 150
SCIENTIFIC SESSION 2
ENVIRONNEMENT
10h45 - 11h00 Guilbaud et al.
11h00 - 11h15 Noel et al.
11h15 - 11h30 Eleloue et al.
11h30 - 11h45 Veilmirovic et al.
11h45 - 12h00 Hayder et al.
12h00 - 12h15 Siripinyanond et al.

12h15 - 13h45 - R0 space
LUNCH

13h05 - 13h45 - room 150
PARTNERS TALKS
Wyatt / Sciex

13h45 - 14h15 - room 150
INVITED LECTURE 1
CHARACTERIZATION AND ENVIRONMENTAL BEHAVIORS OF NANOPARTICLES
Pr. Z. Tan

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SCIENTIFIC SESSION 3
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14h30 - 14h45 Janca et al
14h45 - 15h00 Hoyos et al.

15h00 - 15h30 - R0 space
BREAK

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SCIENTIFIC SESSION 4
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15h45 - 16h00 Ben Azzouz et al.
16h00 - 16h15 Boye et al.
16h15 - 16h30 Contado et al.
16h30 - 16h45 Safenkova et al.
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17h00 - 19h00 - R0 space
WINE/BEER AND CHEESE POSTER SESSION

WEDNESDAY JUNE 5TH

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Pr. Gert Desmet
MACHINE LEARNING FOR LIQUID PHASE SEPARATIONS

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SCIENTIFIC SESSION 5
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9h30 - 9h45 Wahlund P.O. et al.
9h45 - 10h00 Kuklenyik et al.
10h00 - 10h15 Taajiki et al.

10h15 - 10h45 - R0 space
BREAK

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SCIENTIFIC SESSION 6
INSTRUMENTATION DEVELOPEMENT
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11h00 - 11h15 Salmon et al.
11h15 - 11h30 Nilson et al.
11h30 - 11h45 De Carsalade et al.
11h45 - 12h00 Geisler al.
12h00 - 12h15 Graewert et al.

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LUNCH

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PARTNERS TALKS
NanoScale Metrix / Postnova

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INVITED LECTURE 2
RECENT ADVANCES IN TAYLOR DISPERSION ANALYSIS FOR HEALTH APPLICATIONS
Pr. H. Cottet

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SCIENTIFIC SESSION 7
OTHERS TECHNIQUES
14h15 - 14h30 Biagioni et al.
14h30 - 14h45 Ferraro et al.
14h45 - 15h00 Crestani et al.
15h00 - 15h15 Boye, Zielke et al.
15h15 - 15h30 Danusso et al.

15h30 - 16h30 - R0 space - booths
INDUSTRIAL PARTNERS NETWORKING

16h30
SOCIAL PROGRAM
DOWNTOWN HISTORICAL VISIT WITH LVAN

FREE TIME

19h45 - 23h00
GALA DINNER & REVIVAL
BATEAUX NANTAIS

THURSDAY JUNE 6TH

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KEYNOTE LECTURE 3
Pr. Katrina Löchner
FIELD FLOW FRACTIONATION FOR THE ANALYSIS OF NANOPLASTICS IN RELATION TO FOOD SAFETY

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SCIENTIFIC SESSION 8
FOOD
9h15 - 9h30 Rolland-Sabate et al.
9h30 - 9h45 Figue et al.
9h45 - 10h00 Giordani et al.
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BREAK

10h45 - 12h00 - room 150
SCIENTIFIC SESSION 9
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11h00 - 11h15 Bashir et al.
11h15 - 11h30 Podzimek et al.
11h30 - 11h45 Worms et al.
11h45 - 12h00 Ghoshal et al.

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LUNCH

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Shimadzu

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SCIENTIFIC SESSION 10
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13h45 - 14h00 Lopez-Gutierrez et al.
14h00 - 14h15 Rodin et al.
14h15 - 14h30 Giordanii et al.
14h30 - 14h45 Cam et al.
14h45 - 15h00 Jemfer et al.

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SCIENTIFIC SESSION 11
STANDARDIZATION
15h00 - 15h15 Caebergs et al.
15h15 - 15h30 Huber et al.
15h30 - 15h45 Churchill et al.

15h45 - 16h15 - room 150
CLOSING CEREMONY

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Partners Presentations

We are happy to announce that our partners will have dedicated talks to present their latest developments.

Room 150

Tuesday June 4th

13:05-13:25: WATER- WYATT
Enhancing the Eclipse FFF system- with VISION4 software: Comprehensive Control and the Latest Applications.

13:25-13:45: SCIEX
Connecting isoelectric focusing to mass spectrometry using intaBio Imaged CIEF-MS System for Charge Variant Analysis.

Wednesday June 5th

13:05-13:25: Nanoscale Metrix
Size Distribution by Taylor Dispersion Analysis: An Innovation for resolutive size distribution measurement of ultrasmall nanoobjects.

13:25-13:45: Postnova
Latest developments at Postnova

Thursday June 6th

13:10-13:30: Shimadzu
Shimadzu: a universe of innovation and versatility at the service of science.

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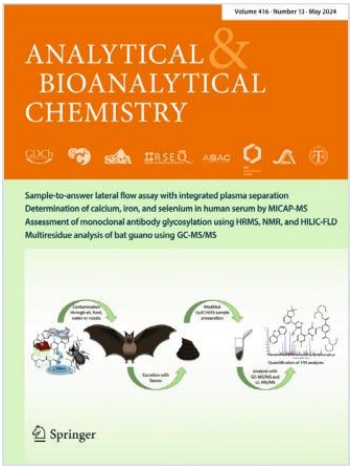

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KEYNOTE LECTURE 1

Comprehensive, size-resolved characterization of pharmaceutical nanoparticles by in-line coupling of AF4 and SAXS

Pr. Heinrich Haas

Nanoparticles are part of various different types of pharmaceutical products. Classically, liposomes have been used for delivery of small molecules for the treatment of cancer or fungal infections. Over the years, many other types of nanoparticle systems and applications were introduced, with lipid nanoparticles of delivery of messenger RNA (mRNA) as the recently most prominent representatives.
Most of the pharmaceutical nanoparticle formulations are polydisperse by their nature.
One key challenge in the development of nano-pharmaceuticals is the control of the size and the size-dependent properties of the nanoparticles.

Our team has coupled asymmetrical-flow field-flow fractionation (AF4), with small angle X-ray scattering (SAXS) measurements to allow for better quantitative characterization of the pharmaceutical products. We used dedicated approaches for data analysis, applying our developed algorithms to match the information from the different detectors. The new instrument set-up allows the quantitative determination of size-resolved structural and quality-related parameters in pharmaceutical nanoparticle products.
The method can be useful for setting up improved quality control procedures, evaluating manufacturing processes during development, or investigating the comparability of products. Additionally, it finds applications in characterizing nanoparticles for toxicological or environmental studies.

Session 1: Diagnostic - part 1

AF4 and orthogonal techniques for mRNA-lipid nanoparticles characterisation

Giuditta Guerrini *[1], Dora Mehn [1], Luigi Calzolai† [1], Sabrina Gioria [1], Diletta Scaccabarozzi [1]

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Physico-chemical characterisation of mRNA-lipid nanoparticles (mRNA-LNPs) is essential both for product development and quality control. As in the case of nanomedicines, the size and stability of the particles and the amount of free *vs.* encapsulated active pharmaceutical ingredient (in this case mRNA) are key quality attributes.

In this work, high quality mRNA-LNPs synthesized with a microfluidic device, were subjected to various stress conditions (sonication and temperature) in order to mimic the unintentional release of mRNA from the particles.

The different samples were analyzed using batch mode DLS AF4-MALS-DLS and analytical ultracentrifugation (AUC). Our AF4 system, using a frit inlet column with 10 kDa regenerated cellulose membrane, was able to separate free mRNA from intact LNPs. Orthogonal data from AUC experiments confirmed the information derived from AF4-DLS data. Results show that AF4 is an excellent technique for measuring the particle size distribution of mRNA-LNP formulations, but also a convenient fractionation device allowing the collection of size fractions for further, off-line analysis.

Investigating the critical quality attributes of lipid nanoparticle prototypes in protein containing media

Rand Abdulrahman * [1], Panida Punnabhum [1], Robin Capomaccio [2], Kevin Treacher [2], Zahra Rattray [1]

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Lipid nanoparticles (LNPs) are emerging new modalities for mRNA therapeutics and have been in the spotlight over the past few years. When prepared synthetically, LNPs were characterised for their CQAs such as particle size, polydispersity index (PDI) and zeta potential (ZP) when dispersed in Phosphate Buffer Solution (PBS) at physiologic pH. Since there is the absence of proteins in PBS, the CQAs of the LNPs might change in the presence of proteins whereby these interactions are essential as they will enable the pre-screening of the LNP formulation as promising therapeutic candidates (3). The aim of this study was to investigate the changes in LNP prototype CQAs in the absence and presence of proteins in the incubation media. PolyA LNPs were manufactured using 1,2-dioleoyl-3-trimethylammonium-propane DOTAP: DSPC: Cholesterol: DMG-PEG2000 at a molar ratio of 50:10:38.5:1.5 mol%. LNP hydrodynamic size was measured using Dynamic Light Scattering (DLS) and Nanoparticle Tracking Analysis (NTA), encapsulation efficiency (EE) and mass balance (MB) were analyzed using the Ribogreen Assay. Further, the same characterisation methods were used to analyse PolyA LNP formulation (1mg/mL) after incubation with 3.5% w/v in Bovine Serum Albumin (BSA) for 2 hours and 24 hours at 37oC. Following the 24 hours incubation of the LNPs with PBS, DLS has shown a size distribution by intensity of two peaks at 98.51nm (intensity 93.1%) and 4227nm (intensity at 3.9%). Comparing this data to the incubation of LNPs with BSA, a peak at 7.561nm (intensity 15.1%) was visible which is that for BSA, and the second peak has shown a higher particle size of 98.51nm (intensity at 93.1%) which suggests that BSA interacted with LNPs and caused a shift in particle size. Corresponding NTA data showed a multimodal distribution with high polydispersity of LNPs due to NP-protein and protein-protein interactions. This work demonstrated changes in the CQAs of LNPs in biologically relevant conditions which provides a more understanding of the fate of LNPs following intravenous administration. Further work is underway to investigate the role of lipid composition in LNP biological stability in complex biological media.

Multi-detector Field-Flow Fractionation for the quantitative characterization of nanopharmaceuticals: from method development to comprehensive data evaluation

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The success of mRNA lipid nanoparticles (LNPs) in the battle against Covid-19 has highlighted the potential of nanoparticle drug products. Besides the recent success story of mRNA- LNPs there are several other platforms that are either already well-established or currently under investigation as potent drug delivery systems. As for other innovative nanopharmaceuticals, the advancement of robust methods and characterization platforms to assess their quality and safety profiles is critical. (1)

To eventually ensure their safe and efficient use, such delivery systems are described by a set of quality parameters generally summarized as critical quality attributes (CQA). These include

e.g. particle size distribution, aggregation behavior, corona formation or payload. There are several analytical tools available to determine these CQA with Field-Flow Fractionation (FFF) among the most promising techniques. (2) Together with regulatory bodies these techniques can help facilitating drug delivery development and clinical translation.

FFF comprises a family of flow-based techniques, where an external force field enables the fractionation of nano-sized sample constituents in suspension. In Asymmetrical Flow FFF (AF4) for example, fractionation by hydrodynamic size is induced by a second flow field. Like in liquid chromatography, FFF can be coupled downstream with multiple detection systems, such as UV/Vis, RI, NTA, MALS and DLS, enabling a comprehensive physico-chemical characterization of nano-sized samples. (3)

We herein demonstrate how software assisted simulation and method optimization can drive innovation towards fast and resource efficient FFF method development. Furthermore, we demonstrate the capabilities for the determination of several CQA of nano-sized drug delivery platforms to underline the potential of multi-detector FFF characterization. Besides a comprehensive data evaluation, we want to highlight the challenges that come with light scattering data treatment. In particular, RNA payload determination of LNP-based vaccines and aggregation behavior of drug delivery systems will be presented and discussed. We showcase pitfalls and necessary steps to obtain comprehensive results.

References:

(1) J.K. Patra, *Journal of Nanobiotechnology*, **2018**, 16:71.

(2) F. Caputo, *Journal of Chromatography A*, **2021**, 1635, 461767.

(3) R. Drexel, *Molecules*, **2020**, 25(20), 4703.

Rapid antimicrobial susceptibility testing by SdFFF directly from positive blood cultures

Linda Tlili [*†1], Audrey Gauthier [1,2], Candice Delagnes [1], Serge Battu [2], Olivier Barraud [1], Gaëlle Begaud[‡2]

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Sepsis is one of the leading causes of mortality worldwide. Studies have shown that the early initiation of appropriate antibiotic therapy is strongly correlated with vital prognosis of patients with bacteraemia. However, conventional antimicrobial susceptibility testing (AST) methods require 16 to 24 hours to provide results from positive blood cultures (PBC). It is therefore a priority to obtain AST results as quickly as possible.
The aim of this study was to evaluate the performance of Sedimentation Field-Flow Fractionation (SdFFF) technology in obtaining rapid AST in less than 3 hours, directly from PBC.

SdFFF is a liquid separation method that allows cell populations to be discriminated according to their physico-chemical characteristics such as size and density. To determine the performance of SdFFF, blood cultures spiked with 50 *Escherichia coli* isolates were used. A calibrated suspension of PBC was incubated without or with 5 different antibiotics at EUCAST 2022 breakpoint concentrations for 2 hours at 37◦C. After centrifugation, bacterial suspensions were injected into the SdFFF machine. Elution signals between untreated and treated samples were compared (PDeltaR and PDeltaAUC, Figure 1) and a threshold allowed classifying bacteria as susceptible or resistant. Results were then compared with the broth microdilution (BMD) reference method.

When the sole PDeltaR was taken into account, results showed that SdFFF has a high categorical agreement, sensitivity and specificity (Table 1) with 9 major errors (ME, strains classified as resistant by the SdFFF method and susceptible by the BMD method) and 1 very major error (VME, strains classified as susceptible by the SdFFF method and resistant by the BMD method). Combination of PDeltaR and PDeltaAUC increased sensitivity (98.2%), specificity (100%) and categorical agreement (99.6%) with only 1 VME. Overall, results are in line with ISO 20776-2:2021 recommendations (CA ≥ 90%; Se and Sp ≥ 95%).
Preliminary results indicate that SdFFF enables rapid AST directly from positive BC with *E. coli* within 3 hours. Trials on a larger number of isolates, bacterial species and antibiotic families will test the robustness and universality of the SdFFF technology.

Session 2: Environment

Nanoplastic characterization in Garonne River by Electrical Asymetrical Flow Field-Flow Fractionation (EAF4) coupled to multi-scale characterization

Léa Guilbaud * [1], Emmanuelle Maria [2], Tiago De Oliveira [2], Magali Albignac [2], Marie Hennetier [1], Louisa Landebrit [2], Frédéric Violleau[1], Gaëtane Lespes [3], Clément Roux [2], Jean François Ghiglione [4], Anne-Françoise Mingotaud [2], Alexandra Ter Halle [2]

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[4] Laboratoire d'Océanographie Microbienne (LOMIC) – Institut National des Sciences de l'Univers, Sorbonne Université, Centre National de la Recherche Scientifique, Observatoire océanologique de Banyuls – Observatoire Océanologique, Banyuls/mer, France

Today's environment is full of polluting plastic debris. These plastics can be subjected to photo-oxidation or mechanical abrasion, forming small plastic particles. Microplastics have been shown to affect both marine and terrestrial organisms. A lot of studies have been carried out on plastic pollution, most of which has focused on microplastics visible to the naked eye (0.3 to 5 mm). Studies on the presence of small microplastics (25 to 300 μm) are rarer, even though they are more numerous and in a significant proportion of total plastic-related pollution.

Nanoplastics are particles derived from the degradation of macro and/or microplastics. They range in size from 1 to 1000 nm and are suspended in a liquid solution (colloidal properties). Nanoplastics can move through organisms and are highly toxic. Their specific physico-chemical characteristics result from a high concentration of atoms on the surface compared with those inside the particle. Thus, changes in surface structure influence the behavior and reactivity of nanoplastics at the nanoscale. In particular, nanoplastics interact with other colloids to form large particle aggregates. Consequently, the study of the fate and behavior of nanoplastics in any natural environment must be correlated with their supramolecular organization.

Rivers are considered as the main carriers of plastic pollution. The aim of our study was to quantify nanoplastics in rivers and understand how they interact with natural colloids. To this end, we isolated the colloidal fraction from Garonne River water and carried out a physico- chemical characterization of this fraction.

Quantification was carried out using Py-GC-MS/MS pyrolysis tandem mass spectrometry. Supramolecular organization was characterized by asymmetric flow (AF4) and asymmetric electric flow (EAF4) fractionation, which was coupled off-line with nanoparticle tracking analysis (NTA), inductively coupled plasma mass spectroscopy (ICP-MS), transmission electron microscopy with energy dispersive X-ray spectroscopy (TEM/EDX) and Py-GC-MS/MS analysis.

Combining molecular-scale characterization with FFF to solve redox mechanisms driving generation and stability of colloids in sediments

Vincent Noel *† [1], Elizabeth Paulus [1], Brandy Stewart [1], Soheyl Tadjiki [2], Samuel Pierce [3], Kristin Boye [1]

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Recurring seasonal wetting and drying of floodplain sediments drives redox processes at solid–water interfaces. These seasonal redox cycling promote shifts in aqueous phase parameters (pH, ionic strength, and ionic composition) and chemical, organic and mineral transformation of the solid phase, which could generate colloids (1nm–1μm), and/or influence colloidal stability. Because they are typically associated with organic matter, micronutrients, and contaminants, colloids may serve as transport vectors throughout redox-affected terrestrial and aquatic systems, impacting ground- and surface-water quality. Despite evidence that redox cycles play a significant role in generation and stability of colloids, the mechanisms, chemical composition, and reactivity/stability of generated colloids are poorly understood.

This lack of knowledge is compounded by challenges associated with the detection and characterization of colloids in natural redox environments, as well the lack of systematic experimental set-up to obtain necessary data to parameterize and validate models to account for colloids. To resolve this knowledge gap, we improved an approach combining our deep expertise of molecular- scale characterization (*e.g.*, X-ray spectroscopy (XAS) and microscopy (STXM)) with field- and flow-based fractionation (FFF). FFF coupled with ICP-MS, UV-, MALS, and zetasizer-DLS detectors separate and categorize particles according to their physico-chemical compositions. Each distinct colloid population are then separately sub-sampled and offline molecular-scale characterized.

Our analyzes reveal that oxic conditions during FFF analyses impact colloid size, we are thus developing a system preserving redox integrity. In parallel, our research corroborates the need and efficiency of FFF-XAS combination. For example, the lab-simulation experiments able to study the impact from redox oscillations on stability of colloids. However, understanding the chemical reactivity/stability of colloids requires tracking newly generated colloids and isolating targeted colloids, using FFF, before characterizing them for chemical and mineral transformations. Inversely, XAS detect the generation of aqueous cluster that are too small to be detected by FFF. Another applicability concerns the challenge posed by the variability in colloids found in natural systems. Painting a clear picture of the molecular-scale composition of each colloid population under environmental conditions is essential to predict their stability and transport. We show that FFF-STXM can successfully separate and characterize different carbon-associated colloids.

AF4-ICP/MS coupling to characterize the affinity of uranium for natural colloidal phases

Candice Eleloue *† [1], Valentin De Carsalade Du Pont [2], Pierre Crançon [1], Pascal Reiller [3], Marc Benedetti [4], Gaëtane Lespes [2]

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The understanding of uranium migration processes from surface soils to the underground environment is crucial in environmental management. The colloidal fraction, especially natural organic complexes (such as humic substances), are identified to play a major role in these processes; the physicochemical characteristics of the environment also intervene.

The ultimate objective of this work is to construct different geochemical models of complexation and transport of uranium by natural colloidal humic substances, based on experimental characterizations of soil matrices. To achieve this, exchanges between colloidal, dissolved, and solid phases were considered, taking into account the specificities of humic substances, in the presence of uranium. Sorption isotherms were also constructed to acquire the indispensable data for the construction of geochemical models.

To achieve our experimental objectives, a multi-technique analytical strategy centered on the coupling between AF4 (Asymmetric Flow Field-Flow Fractionation) and Inductively Coupled Plasma Mass Spectrometry (ICP-MS) was implemented. In this presentation, we will first address various methodological points inherent to the fractionation of colloidal phases carrying trace elements and the quantification of these elements. We will then show how the AF4/ICP- MS coupling has allowed access not only to the distribution of uranium in the colloidal size continuum but also to the thermodynamic constants explaining uranium behavior towards organic matter.

Detection and Characterization of Nanoplastics in Fish Samples: A Comprehensive Multimethod Approach

Milica Velimirovic ∗ 1, G´eraldine Dumont 1, Xiaoyu Zhang 1, Kristof Tirez 1, Caroline Geeraerts 2, Claude Belpaire 2, Stefan Voorspoels 1

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[2] Research Institute for Nature and Forest (INBO) – Dwersbos 28, 1630 Linkebeek, Belgium

The analysis of nanoplastics (NPs) in fish samples has gained increasing importance due to growing concerns about their environmental and health impacts. In order to detect and completely characterize NPs in fish samples a multidimensional method for nanoparticle analysis would be required.

This study, conducted within the framework of the MS4Plastics project (H2020-MSCA-IF-2020 - Grant Agreement No 101023205), explores different sample preparation procedures and possibility of using asymmetric flow field-flow fractionation (AF4) coupled with multi-angle laser light scattering (MALLS), and consequent pyrolysis–gas chromatography–mass spectrometry (Py-GC/MS) for the comprehensive characterization of NPs spiked in fish samples. The sample preparation procedure was evaluated based on pre-defined quality criteria, which are mass recovery and change in particle size distribution.

The separation of NPs from the spiked fish samples can be achieved by enzymatic digestion and/or H2O2 digestion without significant alteration in the particle size distribution. The use of AF4-UV/VIS-MALLS allows for the separation and size fractionation of NPs, while Py- GC/MS complements the characterization by offering insights into the chemical composition of NPs.

The developed method offers enhanced capabilities for the analysis of NPs, addressing the growing concerns regarding these contaminants. It represents a valuable tool for researchers and environmental analysts to assess the impact of NPs in fish samples, contributing to a better understanding of the potential risks associated with nanoplastics in aquatic ecosystems.

Towards simultaneous size determination and polymer identification of nanoplastics in meso- and macrocosms

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With constantly growing plastic production, plastic pollution has now become a major environmental issue. Especially minute particles resulting from plastic degradation are hazardous. It is now clear that nanoplastics (< 1000 nm) are omnipresent in all environmental compartments, hence the urgent need of accurate analytical methods for their screening. However, nanoplastics characterization is still immature (Mitrano et al., 2021).
One technique allowing to obtain information on sizes of plastic nanoparticles is Asymmetric Flow Field-Flow Fractionation with Multi-Angle Light Scattering detection (AF4-MALS) (El Hadri et al., 2021). This approach has already been suggested for nanoplastics characterization, however, has mostly been applied in proof-of-concept experiments carried out on simple standard mixtures rather than on realistic samples. Therefore, we aim to validate the applicability of AF4-MALS for nanoplastics analysis in highly complex environmental samples.

To fulfill our aim, we use AF4-MALS for size characterization of nanoplastics present in wastewater treatment plant effluents. The same approach is applied to samples of fresh- and seawater from a laboratory-controlled mesocosm. We identified multiple difficulties in pretreatment and analysis of samples. For nanoplastics coming from the environment, the main challenge is to achieve a sufficiently low limit of detection, compatible with particle concentrations in the samples. By adding an in-line preconcentration step, we managed to achieve detection limits reaching nanograms per liter and a continuous size distribution down to 150 nm. We also elucidate the potential of coupling the AF4 separation with other analytical techniques applied on the same samples: (Dynamic Light Scattering (DLS) for further characterization of the particles, and pyrolysis GC-MS (pyGC-MS) for plastic identification. However, this combination with AF4 poses additional challenges, which we also address.

Our results suggest that using both AF4-MALS and pyGC-MS techniques together results in versatile and complementary information as related to size and chemical composition on the nanometer-sized plastic particles present in the environment from diverse sources. So far, no direct AF4-pyGC-MS coupling has been described. Our research contributes to achieving this, providing the community with an advanced and comprehensive tool for nanoplastic analysis, and thus to properly evaluate the emerging.

Use of Field-Flow Fractionation and Inductively Coupled Plasma Mass Spectrometry for Tracking Nanoparticles During Synthesis and Colorimetric Sensing

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Field-flow fractionation (FFF) and inductively coupled plasma mass spectrometry (ICP-MS) were used for tracking changes of nanoparticles during their synthesis and colorimetric sensing of analytes. FFF-ICP-MS and single particle ICP-MS (SP-ICP-MS) offer various information for nanoparticles including diameter for monometallic nanoparticles; core size and shell thickness for bimetallic core-shell nanoparticles(1), and equivalent spherical diameter, edge length, and thickness of triangular silver nanoplates(2). Then, FFF and SP-ICP-MS were used to track changes of triangular silver nanoplates during their synthesis and colorimetric sensing of mercury ions through anti-etching mechanism from bromide ions(3). SP-ICP-MS offers the information on equivalent spherical diameter and the numbers of particle which were used for understanding the sensing mechanism of the silver nanoplates towards mercury ions. FFF-ICP-MS was employed to observe the association between silver and mercury ions. The fractograms from FFF-ICP-MS showed no association of Ag with other metal ions (Cu2+, Ni2+, Co2+, Cd2+, Pb2+, and Mn2+), confirming the selectivity of silver nanoplates towards mercury ions. This study shows potential application of FFF-ICP-MS and SP-ICP-MS for quality control of nanoparticles synthesis and also for research and development of nanoparticle sensors.

Keywords: Silver nanoplates; Bimetallic nanoparticles; Field-flow fractionation; Single particle ICP-MS; Colorimetric sensor; nanoplastic pollution problem.

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INVITED LECTURE 1

Studies on characterization and environmental behaviors of typical nanoparticles by using field-flow fractionation

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With the widely use of nanomaterial products, a large number of nanoparticles are inevitably released into the environment, and their environmental health risks have gradually attracted attention in recent years. Numerous studies have confirmed that the physical and chemical properties, environmental behavior, and toxic effects of nanoparticles are closely related to the factors such as concentration, size, and composition. Due to the lack of effective separation and detection methods, research on the environmental and toxic effects of nanoparticles is mainly carried out in simple matrix using high concentrations of a certain sized nanoparticles, which fails to accurately illustrate the environmental behaviors of nanoparticles under realistic conditions. To solve the problem, the hollow-fiber flow field-flow fractionation was hyphenated with an inductively coupled plasma mass spectrometer to develop a separation, identification, and quantitative analysis method for particulate and ionic silver species. Based on the analytical platform, we further studied the generation and transformation behavior of low concentration silver nanoparticles in environmental media. In addition, the hollow-fiber flow field-flow fractionation was coupled with a point discharge optical emission spectrometer to establish a new method for the separation, identification, and quantification of different-sized polystyrene nanoplastics, and further study their interaction with natural organic matter. The above research provides important technical support for revealing the environmental behavior and biological effects of typical nanoparticles in realistic environmental media.

Session 3: Theory

Simultaneous programming of field strength and flow rate in field-flow fractionation: a theoretical examination

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The advantages of field programming in FFF are well known. It facilitates the analysis of a wider range of particles sizes that can be obtained at constant field. This is the result of the controlled reduction of the selectivity of the separation, which can be excessive at constant field. The increase of flow rate during sample elution has also been considered for hastening the migration along the channel of the more retained fractions. The simultaneous programming of both field strength and flow rate has also been implemented in the laboratory for both sedimentation and symmetrical flow FFF with promising results. Asymmetrical flow field-flow fractionation (AsFlFFF or AF4) can also be run with programmed decay of cross flow rate through the membrane, but the channel outlet flow rate is usually held constant. Some experimental work has involved the programmed increase of outlet flow rate with time but with fixed cross flow rate. In this work, the simultaneous programming of cross flow rate and channel outlet flow rate is considered. The use of mathematical modeling can reveal the influence of various field decay functions combined with various channel flow rate functions on the elution of particulate samples. (In the case of AsFlFFF, the programmed increase of outlet flow rate will be considered.) The equations used in the calculations will be briefly presented, and simulated fractograms will also be shown to illustrate the predicted effects.

On the Focusing Mechanism in Thermal Field-Flow Fractionation of Macromolecules

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In 1988, Giddings et al. published a paper (1) in which they claimed the first" entropy- driven" hyperlayer FFF of ultra-high molar mass (UHMM) polystyrenes (PS) in Thermal Field- Flow Fractionation (TFFF). In 1992, Janča and Martin (2) studied the influence of the operational parameters on the retention in TFFF of UHMM PS of similar molar masses as used by Giddings (1).

If the flow of the carrier liquid is not stopped for a time necessary to establish the initial steady- state distribution of the sample across the channel thickness, a serious zone broadening and false retention occur. The starting position of the separated species must imperatively be at the accumulation wall (3).

In our recent paper (4), two fundamental mechanisms of focusing in Micro-TFFF are analyzed from the point of view of modern thermodynamics. Two books (5,6), published by prominent scientists, consider the entropy as the product of reversible or irreversible processes. A simple expression:

$$diS = FdX \quad (1)$$

defines that the change of entropy diS of an irreversible process is the product of the thermodynamic force F that produces the flow dX of a quantity such as heat or matter. For example, the interaction between the heat and matter produces Soret effect when heat flow drives a flow of matter, and Dufour effect when the concentration gradient drives a heat flow, both effects resulting in entropy production. According to this understanding of the second law of the thermodynamics, the entropy is a product but not the driving force.

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Guided self-organization and complexity in field-flow based separation techniques

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Separation of species under flow implies a transition of a mixture from a certain entropy state at the beginning of the separation process to another state with lower entropy at the end of the process. As stated in the second law of thermodynamics, reducing entropy requires adding energy to the system and generating the work required to increase the macroscopic order. Self-organization is characterized by the same process. Contrarily to self-organization in case of biological cells leading to forming new structures without any external mechanism from an aggregate of very well determined molecules, in separation phenomena (like in FFF), separation implied reduction of entropy is guided by an external field force. However, organized dynamical structures can be induced during a separation process. In the transition disorder-order, self- organization we may define the complexity in agreement with the modern tendency of using complexity for analyzing collective phenomena. The main goal of this work is, by using some examples, to propose to introduce in separation sciences self-organization and complexity as elements of a new way of analyzing the field-flow based separations. Finally, as we will try to demonstrate, we could link self-organization to selectivity in FFF, while complexity can be linked to resolution.

Session 4: Polymers - part 1

Introducing TGE - 3DCoThFFF: The case of analyzing photoactive analytes in heterogeneous systems

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Analysing multicomponent and heterogeneous systems is extremely challenging in thermal field-flow fractionation (ThFFF), particularly when it comes to achieving optimal separations and characterizations while utilizing isothermal conditions. In response to this challenge, we propose an innovative analytical approach for photoactive analytes, termed temperature-gradient- elution (TGE) combined with 3-dimensional correlation ThFFF (TGE-3DCoThFFF). This sophisticated strategy involves correlating data obtained from multidetector ThFFF within a 3-dimensional framework, providing an advanced analytical method for tracking microstructural dynamics. For proof of application, we hyphenated ThFFF with UV-Vis photo-spectroscopy, multiangle light scattering, dynamic light scattering, viscosimetry and differential refractive index detectors; and this approach is demonstrated on styrene - maleic anhydride copolymers and styrene - ethylene oxide nanostructures.

Asymmetric flow field-flow fractionation coupled with inductively coupled plasma mass spectrometry (A4F-ICP/MS) as a control quality technique to characterize the grafting state of polystyrene onto gold nanoparticles

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Nanoplastics are omnipresent not only in the environment, but can also be transmitted to humans via the food chain or by contaminating respiratory air. These pollutants have the ability to cross biological barriers, carried into the bloodstream and reach different organs such as the placenta and compromise the fetal development (1). The ability to localize and quantify nanoplastics in human tissues is crucial to understand the mechanisms of their uptake and transport into cells and better assess their potential risks. Electron microscopy (EM) represents an exceptional method to study the uptake and intracellular fate of nanoparticles. However, polymeric materials are more difficult to visualize in cells because they have low contrast, and are mainly composed of chemical elements that can be confused with cellular constituents (2). The use of metal-polymer hybrid nanoparticles constitutes a real solution to solve the problem of low electronic contrast of polymer systems.

Here, 20 nm gold-polystyrene (Au-PS) nanoparticles were synthesized as a model of metal- polymer hybrid nanoparticles to visualize nanoplastic-like particles in cells. As non-manufactured products, the characterization of these assemblies was carried out before cell contact. In this talk, the capability of asymmetric flow field-flow fractionation coupled with inductively coupled plasma mass spectrometry (A4F-ICP/MS) will be presented as a control quality technique to characterize the grafting state of polystyrene onto gold nanoparticles as well as the size and concentration of the synthetized nanoparticles.

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Beyond Size – How AF4 with multidetection How AF4 with multidetection can contribute to a better understanding of polymeric vesicles

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Carrier systems (for drugs, enzymes, etc.) based on water-soluble, stimuli-responsive polymer vesicles offer a variety of possibilities. First and foremost is the ability to adjust and individualize their properties. The chemistry toolbox enables the design of a polymersome where size, membrane thickness, stability, loading, and release properties can be customized through the selection of block copolymers and modifications.

However, the process of formation and loading is influenced by many different parameters such as concentration, pH value, temperature, and purification. Therefore, certain analytical steps must be applied. This can be time-consuming. In addition, the high complexity can lead to misinterpretation of data obtained especially with batch methods. For this reason, we have developed a procedure for the comprehensive characterization of polymersomes using AF4 with multi-detection. Many advantages are exploited, such as the large separation range, and the combination of concentration (RI and UV) and size sensitive detectors (SLS and DLS). With the large amount of data, we can draw conclusions about size and molar mass distributions. In addition, by looking more closely at the conformational properties, we can make statements about the extent to which, for example, purification processes work or better understand the effectiveness of purification processes. In this talk we will introduce our protocol and present its capabilities. This includes the confirmation of the spherical shape, its change depending on the loading of different cargoes and the influence of the block copolymer composition on the conformation of the polymersomes.

For the first time, we have coupled the AF4 with SAXS to gain an even deeper insight into the structural properties and the first results will be presented here.

A new challenge for the Centrifugal Field Flow Fractionation: the separation of poly(N-isopropylacrylamide) microgels

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Nanogel and microgel based on poly(N-isopropylacrylamide) (PNIPAM) are a highly researched type of colloidal material, since they exhibit reversible volume-phase transition (swelling– deswelling volume change) in water, responding to external temperature change around the lower critical solution temperature (LCST), which is close to human body (1, 2).
When PNIPAM microgels are used in studies such as cell-surface interactions or carrier for controlled drug release, along with many other physical and chemical parameters, the microgel particle size is an important parameter to consider (3).

In the FFF family, the asymmetric-flow field-flow fractionation (AF4) is used to sort and provide high resolution particle size distribution and structural information about this kind of samples
(4). In this work, the Centrifugal Field-Flow Fractionation (CF3) is proposed, for the first time, to study different PNIPAM microgels in the size range 100-1000 nm (hydrodynamic diameter). The most critical aspect to correctly fractionate this kind of sample is the microgel density, close to the water density. Particles were at first analysed by DLS and TEM and then the CF3 was used to investigate how the separation conditions impact on the estimated sizes. Different centrifugal fields and carrier compositions were tested in order to select the most convenient fractionation conditions.

To improve the detectability, “staining” with gold nanoparticles (AuNPs) has been proposed. By mixing the PNIPAM microgels and the AuNPs in different volume ratios, it has been proven that when the relative particle sizes between the two colloidal systems is close to 1/3 the resulting assembling can be properly separated and revelled. AuNPs stabilized with cysteamine or sodium citrate were both prepared, characterized and tested and only the ones with cysteamine were found to be suitable to the proposed aim because of the right combination between the ionic charges on the surface of the PNIPAM and AuNPs particles.

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Asymmetric flow field-flow fractionation for receptors-based recognition of specific compounds in complex samples

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The main trend for detection of specific compounds in complex samples is to integrate their receptors-based recognition with the use of different labels and/or separation techniques. The presented study demonstrates efficiency of asymmetric flow field-flow fractionation (AF4) as a tool to register formation of analyte–receptor complexes directly in the reaction mixture. The investigations of immune complexes and products of nucleic acids amplification were implemented using the AF4 platform with Wyatt Eclipse 3+, Dawn HELEOS II detector, Optilab T-Rex refractometer (Wyatt Technology, USA), and 1260 Infinity LC System with variable wavelength detector (Agilent Technologies, USA).

The tested antigens were lipopolysaccharides (LPS) from *Salmonella enteritidis.* Their heterogeneity was demonstrated by dynamic light scattering (DLS) and agarose gel electrophoresis. Under the optimized liquid flow regime of the AF4, multi-angle laser light scattering (MALLS) and DLS demonstrated four peaks with radii from 150 to 300 nm in the bulk preparation, while DLS in batch mode recorded only one broad peak. The addition of monoclonal antibodies to the LPS (HyTest, Russia) provided the shifts of MALLS and DLS peaks for complexes of lipopolysaccharides with the antibodies.

Next, mixtures of DNA amplicons obtained by loop-mediated isothermal amplification (LAMP) of target gene of *Salmonella* spp. were controlled by the AF4 registering absorption at 260 nm. This signal from the amplification products showed several peaks. The inclusion of tags (such as biotin, fluorescein) into the LAMP primers and addition of tag-binding receptors (streptavidin, antibodies to fluorescein) led to movement of specific AF4 peaks. These results assist in the choice the best primers for labeling as well as the amplification time.

Thus, the demonstrated efficiency of the AF4 technique confirms its potential applicability for different tasks associated with rapid non-separating recognition of specific compounds in complex samples.

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Elucidating the Secrets of Soluble Extracellular Polymers in Algae Cultivation using AF4-UV-MALS-dRI

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Extracellular polymeric substances (EPS) from algae are complex, secreted, soluble heteropolymers (comprised of carbohydrates and proteins), which can represent a significant fraction of biological extracellular carbon. While it remains unclear whether EPS is deliberately produced or a product of excess carbon secretion, these polymers play a tremendously important role in antiviral and antimicrobial bioactivities *in vivo*. These bioactivities are often controlled by molecular properties, such as composition, molecular weight (MW), size, and charge. The structure-function relationship of these complex polymers has yet to be established due to their heterogeneity, high MW, high charge density, and native high-ionic strength environment, all of which make analysis difficult. To meet this analytical challenge, a multi-faceted approach is required. In this study, AF4-UV-MALS-dRI was used to fractionate and evaluate the different size populations present in the EPS of *Chlorella vulgaris.* Fractions were collected and analyzed to probe compositional differences using high-performance anion exchange chromatography (HPAEC), and liquid chromatography-mass spectrometry (LCMS). The study uses AF4 to investigate the effects of the ionic strength and composition of the carrier fluid on the retention and aggregation behavior of these biopolymers to better understand their interactions in their native, higher-salinity, environments. The AF4 results demonstrated that the EPS of *C. vulgaris* has three populations spanning a molecular weight range from 4 x 10ˆ4 – 3 x 10ˆ8 Daltons. Offline compositional analyses of the primary structures revealed heterogeneity in monosaccharides and amino acids between the different size populations. Distinct ion-mediated behaviour was observed among the fractions in the different ionic environments revealing EPS retention was affected by both ionic strength and the chao-/kosmotropicity of the electrolytes. We report for the first time that *Chlorella* cultures exhibit different isolated polymers, with distinct primary, secondary, and tertiary structures, and MW distributions, suggesting distinct biological functions among the polymer distributions. With the unique application of AF4 presented here, it may become possible to probe the fundamental biological role of each polymer population over time and their role in sustaining a healthy ecosystem.

KEYNOTE LECTURE 2

Machine Learning for Liquid Phase Separations

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Due to its versatility in solving various types of problems, artificial Intelligence (AI) and machine learning (ML) are penetrating literally every domain in science and technology (healthcare, finance, manufacturing, natural language processing, marketing, …). And obviously also separation science makes no exception to this and can benefit from the modelling and search capabilities of AI.

The present contribution will first introduce the key principles of some of the most important ML techniques: neural networks, Bayesian optimization and reinforcement learning. Next, a number of applications of ML in separation science, such as the prediction of retention time in liquid chromatography, will be shown.
Most attention will be given to the solution of an optimization problem where the shape of the micro-pillars used in a micromachined separation column (on-chip device for liquid (hydrodynamic) chromatography) is optimized such that the pillar bed provides the best possible compromise between longitudinal mixing and flow resistance. Whereas cylindrical pillars are the most obvious choice to fill the bed, there are pillars with a more ellipsoidal or elongated diamond shapes that provide a significantly better compromise between longitudinal mixing and flow resistance. It will be demonstrated how Bayesian optimization can guide the search process leading to the most optimal shape.

Session 5: Pharmaceuticals

Physicochemical characterisation of biodegradable and tuneable polymeric nanoparticles.

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Polymeric nanoparticles are solid colloidal particles with a size in the range of 10–1000 nm and are made of biodegradable and biocompatible polymers or copolymers, in which the drug is entrapped or encapsulated. These nanocarriers have been designed with complex materials and structures providing them critical quality attributes (CQAs). These CQAs are characterised and controlled during the manufacturing process to ensure the safety and the efficacy of the drug for the patient.

AstraZeneca has developed AZD2811 nanoparticles made of polylactic acid–polyethylene glycol (PLA–PEG) and encapsulating an inhibitor of Aurora B kinase controlling the cell cycle. Preclinical studies on AZD2811NPs showed continuous drug release over weeks in-vitro and in plasma concentrations in rats with a reduction in tumour volume.1,2

In this context, we applied an extensive physicochemical characterisation of input material and particles using variety of orthogonal macromolecular and colloidal techniques. The resulting data will be presented in the context of utilising the most appropriate techniques to fully understand and control the manufacturing and performance of AZD2811 nanoparticles.

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AF4 as Predictive Assay for Antibody Self-Association

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Monoclonal antibodies (mAbs) formulations usually require high-protein-concentration solutions, which can exhibit physical stability issues such as high viscosity and opalescence. To ensure that mAb-based drugs can meet their manufacturing, stability, and delivery requirements, it is advantageous to screen for and select mAbs during discovery that are not prone to such behaviors. These macroscopic properties can to a certain extent be assessed from the diffusion interaction parameter (kD) obtained by dynamic light scattering. kD which is a measure of self- association under dilute conditions, can be challenging to measure at the early stage of discovery, where a large amount of a high-purity material, is often not available. An alternative method to screen biopharmaceutical molecules for protein-protein attraction at high concentration utilizes the exceedingly high concentrations that occur close to the membrane in an Asymmetric Flow Field Flow Fractionation (AF4) measurement. The results are insensitive to the concentration or buffer composition of the sample solution and only depend on the absolute amount of protein loaded and on the running buffer. This makes the method extremely useful in a compound discovery workflow and to assess developability. Extensive studies on various mAbs show that the measured retention time of the antibodies allows us to pinpoint molecules that exhibit issues at high concentrations. Remarkably, our AF4 assay requires minor sample amount (30 μg) under dilute conditions and does not need extensive sample purification.

Comparison of lipoprotein particle composition in cerebrospinal fluid and plasma using AF4-LC-MS/MS

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Background: Although plasma lipoproteins have been extensively studied for size, protein, and lipid composition, much less is known about lipoprotein particles in the brain. In this work we investigated the composition of lipoprotein particles that contain apolipoproteins (apos) A1, A2 and E in cerebrospinal fluid (CSF) compared to plasma.

Methods: The composition of CSF and plasma lipoproteins in different size ranges was determined by asymmetric flow field flow (AF4) fractionation, coupled with downstream analysis of main protein and lipid constituents by liquid chromatography and mass spectrometry (LC/MS). In each fraction, a panel of protein and lipid constituents were quantified, including the relative abundance of lipid species within phospholipid (PL) classes. The calculated composition measures included lipid/protein molecular volume ratios, core-volume/surface-volume ratios, and number of molecules per particle.

Results: In CSF, we found apoA1/A2-, apoA1/A2/apoE- and apoE-containing particles, in 8-11 nm, 11-15 nm and 15-20 nm, carrying different sets of other proteins. In number of particles, the apoA1/apoA2/apoE containing particle were the main lipid carriers in CSF, unlike the apoA1/apoA2 particles in plasma. In comparison of corresponding size ranges, CSF-HDL particles grow in particle size by gaining a thicker protein enriched surface, while plasma-HDL particles grow by gaining a larger core, inherent in the structure and lipid-binding properties of apoE molecules compared to apoA1 and apoA2. Using same sample sets, the AF4-LC-MS/MS- derived particle number profiles are compared with those obtained by nuclear magnetic resonance and ion mobility analysis.

Conclusion: We reveal novel properties of CSF lipoproteins, in terms of particle distribution by size, composition and aggregation propensity with relevance to AD pathogenesis.

Centrifugal FFF as a femto-balance for the measurement of mass and protein cargo of drug delivery vehicles

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Lipid nanoparticles (LNP) and exosomes have recently become the focal point of nanomedicine and personalized treatment. Their biocompatibility, protective nature, and controlled release capabilities make them great candidates for drug delivery and personalized medicine. A thorough understanding of the physicochemical properties of LNPs and exosomes, particularly their mass, size, and drug payload, is critical to ensure synthesis reproducibility, stability, and therapeutic effectiveness.

It has been demonstrated that Centrifugal Field-Flow Fractionation can measure nanoparticle mass (1). This study used the same methodology to measure the buoyant mass of a single LNP or exosome directly at the femtogram level. The measured buoyant mass values were transformed into actual mass values using Archimedes' principle and the particle volume. Particle volume was taken from the manufacturer's data or determined experimentally using light scattering or electron microscopy. The average mass of the payload per vehicle was measured as the mass difference between empty and full vehicles.

This method provides a novel and simple method to determine the size and payload of the drug delivery vehicles. The full methodology, advantages, and limitations will be discussed.

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Session 6: Instrumentation development

Particle Density Determination Using Resonant Mass Measurement Method Combined with Asymmetrical Flow Field-Flow Fractionation Method

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A novel characterisation system using a combinational analysis of the resonant mass measurement (RMM) and asymmetrical flow field-flow fractionation (AF4) methods is developed as a hybrid analytical tool for the particle density of mixtures of different-sized materials. The function of the RMM method is to determine the particle mass by observing the shift in frequency proportional to the particle mass. However, to determine the density of particles using the RMM method, information on the size or size distribution is necessary. Because the size distribution of the particles could influence the accuracy of the determination of the density of the particles, this study addresses the weak point of the RMM method using the AF4 method. First, AF4 is used to fractionate the narrow-sized distributed particles as an effective sample preparation method before the RMM assessment. Moreover, the accurate size distribution determined by the AF4 method with multi-angle light scattering analysis supports the reliable density determination by the RMM method on the transformation from the mass distribution of the particles to the density distribution. Using our developed combinational analytical method of RMM and AF4 methods for mixed particle samples (different sizes and different materials), the densities of the respective particles are evaluated.

Development of a miniaturized asymmetrical flow field flow fractionation on a soft thermoplastic chip

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Though AF4 has been around for years, its use continues to spectacularly spread in other fields such as biotherapeutics, changing the characterization standard and our interaction with nanometric items one of the main reasons being that it overcomes the limits of chromatography. Yet, the technique is limited by several important factors: complexity, time, quantities used and cost. This might at least partially explain the slow diffusion of the technique in time, particularly for academia and biotechnology laboratories. Miniaturization has shown to address these limiting factors but the current assembly method (screw clamping) intrinsically limits the possibilities of design. Microfluidics has led to a change of paradigm for prototyping and developing analytical techniques, allowing to miniaturize while intensifying separative and detection methods in particular. Our group is developing a miniaturized AF4 microfluidic prototype based on a soft thermoplastic elastomer encapsulating an ultrafiltration membrane. The device is fabricated by standard lithography technique and hot embossing, it is easy and fast to produce as well as relatively inexpensive. The thickness of the channels are below 100um and can be tailored to the application targeted. We have demonstrated the sealing of the membrane at room temperature (no burst observed below 2 bars) and compartmentalization of nanometric (100nm) fluorescent beads in the flow channel. The hydrodynamic and thermal control of the uAF4 device is simplified compared to the macrometric one and our prototype is compatible with inverted epifluorescence microscopy. By demonstrating the different operating modes of AF4, the uAF4 device could remove a technological barrier for users as well as improve the performance of separation and potential for biotherapeutis and nanopharmacology, where samples quantities can be limited.

In-line coupling of asymmetric flow field-flow fractionation (AF4) to synchrotron small angle x-ray scattering (SAXS) for characterization of proteins and bionanoparticles

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SAXS is routinely used for the characterization of conformational properties hierarchical structures in proteins and nanoparticles. As with all batch-based characterization techniques, challenges and limitations arise when characterizing multi-component and/or polydisperse samples with SAXS, as average properties are obtained. By coupling of a fractionation technique, such as AF4, prior to SAXS characterization the complexity is reduced and size-resolved SAXS data can be obtained over a wide size range. This coupling has a large potential for applications in the life sciences, as it opens the possibility to characterize sensitive and/or complex samples as well as samples with a broad size distribution. For low electron density samples, e.g. biomolecules and biologics, high x-ray flux is necessary and, hence, synchrotron SAXS is utilized.

In this presentation, examples of characterization using AF4 coupled to synchrotron SAXS at coSAXS beamline at MAX IV, Lund, Sweden will be shown for proteins and nanoparticles intended for therapeutic applications, including lipid nanoparticles for delivery of biologics. Furthermore, limitations and challenges in the AF4-SAXS coupling will be briefly discussed.

Potentiality of cyclical electrical field-flow fractionation for zeta potential measurement in environmental conditions

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The zeta potential is a crucial parameter to evaluate the stability of particles in suspension and determine their behaviour in the environment. It strongly depends on the medium characteristics (pH; ionic strength). However, the requirement of some techniques like zetasizer might not be adapted to measure the zeta potential in environmental conditions, especially in (sub)surface waters with low ionic strength. Among field flow fractionation techniques, cyclical electrical field-flow fractionation (Cy-ElFFF) fractionates analytes only in function of their electrophoretic mobility by applying a cyclical electrical field in the channel. Electrophoretic mobility depends on the electric charge on the surface of the particles; this electric charge also determines the zeta potential. The relationship between electrophoretic mobility and zeta potential is well known. Therefore, the measurement of the first one enables the second to be determined. Thus, the zeta potential of an analyte can be determined with a simple measure of its retention time in Cy-ElFFF, by external calibration with standard nanoparticles. In this talk, the capability of Cy-ElFFF to determine zeta potential of manufactured nanoparticles (MNPs) in media of very low ionic strength will be presented. Samples containing MNPs of different chemical compositions, with and without coating and/or of different sizes were analysed. The results obtained will illustrate how Cy-ElFFF can be positioned as a new way of measuring zeta potential, fully complementary to those already existing.

High Temperature Thermal Field Flow Fractionation: Meeting the Challenges in Advanced Characterization of Polyolefins

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Polyolefins (PO) are widely used synthetic polymers in industry due to their exceptional properties, leading to applications, from packaging to durable, high-impact structural components. However, characterizing them has remained a great challenge due to their complex molecular structures comprising long chain branching as well as in some cases ultra-high molecular weights (UHMW). Any analytical as well as fractionation method needs to be performed at high temperatures due to their insolubility at ambient conditions. State-of-the-art separation methods, such as High-temperature size exclusion chromatography (HT-SEC) or interaction chromatography (HT-IC) are limited in their ability to provide a sophisticated analysis due to insufficient separation range or coelution of differently branched fractions (1).

Field-flow-fractionation offers a great alternative for the separation of complex polyolefin and overcomes the problems seen in HT-SEC. The feasibility of using high temperature thermal FFF (HT-ThFFF) to separate polyethylene was demonstrated over 40 years ago (2). Only HT-AF4 has been applied thus far to characterize differently long chain branched PO and UHMW PO

(3). Despite this advancement, HT-AF4 remains unable to separate differently branched PO having similar hydrodynamic volumes. ThFFF instead may overcome this limitation because its separation mechanism is intrinsically sensitive to branching, as recently shown (4,5).

Here, we demonstrate the feasibility of using HT-ThFFF hyphenated to information-rich detectors such as light scattering and viscometry as a separation and branching characterization technique for PO. To achieve this, we have redesigned HT-ThFFF with modern design and construction and implemented it in an established HT-environment. Our mid-term goal is to integrate HT-ThFFF into the future routine analysis of PO commodities.

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FFF-to-SAXS: Multifaceted Characterization of Nanoparticles and Proteins

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Biological Small-Angle X-ray Scattering (SAXS) is a powerful analytical technique for studying the structural and biophysical properties of biological pharmaceuticals and various nanoparticles at the nanoscale level. SAXS offers versatile insights into many aspects of biological pharmaceuticals, including oligomeric compositions, stability assessment, interaction studies, and flexibility analysis. For instance, SAXS has been instrumental in determining the oligomeric compositions of insulin formulations, assessing the stability and quality control of mRNA-LNP formulations, understanding the binding interactions between antibodies and the SARS-CoV-2 spike protein, and evaluating the flexibility of protein-protein conjugates.

To enhance the resolution and accuracy of SAXS analysis, we have developed a novel approach by coupling SAXS with Field-Flow Fractionation (FFF). This combined FFF-to-SAXS technique enables the characterization of individual components within complex samples, providing detailed structural information with improved precision. Current applications of this technique include quantitative size-resolved characterization of mRNA nanoparticles structural analysis of protein samples (Figure 1) separation and characterization of nanoplastics. Notably, interested researchers may access this cutting-edge SAXS instrumentation through the European Molecular Biology Laboratory (EMBL). EMBL's bioSAXS beamline P12 serves as a nexus for innovative structural biology research and is situated at the PETRA III synchrotron facility @ DESY (Deutsches Elektronen- Synchrotron in Hamburg, Germany). Supported by funding from the German Federal Ministry of Education and Research (BMBF # 05K22UM3), this collaborative effort aims to advance our understanding in structural biology, biopharmaceutical innovation, and environmental research.

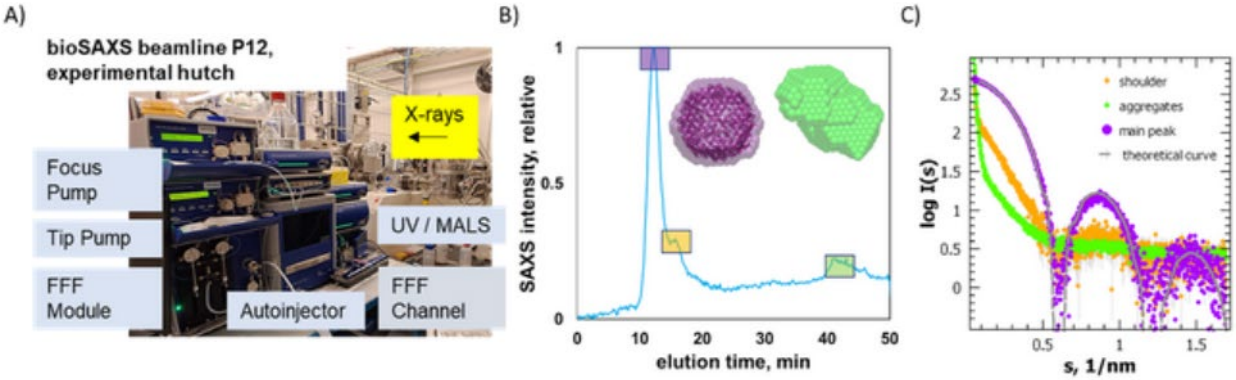

Figure 1: FFF coupled SAXS apparatus and data on ferritin (nanocarrier). FFF module inside the experimental hutch of the P12 bioSAXS beamline in Hamburg, Germany. (A) SAXS fractogram of the protein ferritin. 3 regions of interest are marked and corresponding SAXS scattering profiles are shown in C). Inserts show selected SAXS envelopes. (B) SAXS profiles are shown as log I(s) vs (s), whereby s = 4πsin theta/λ, and 2 theta is the scattering angle. In addition to information on shape and size (radius of gyration, maximum distance) the degree of flexibility can be retrieved from the data.

Session 7: Other techniques

Recent advances in Taylor dispersion analysis for health applications

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Taylor dispersion analysis (TDA) is a new promising technique for the determination of diffusion coefficients and hydrodynamic radii of a myriad of nanoscale objects. The principle of this method is based on the band broadening of a solute plug injected in a miniaturized Poiseuille flow (50 µm i.d. capillaries). It allows determining the hydrodynamic radius of virtually any mixture of solutes, on a range of size ranking between 0.1 and 300 nm. In this presentation, after a brief introduction about the principle of TDA, different applications will be presented including the characterization of mRNA loaded lipidic nanoparticles and their formulations (LNP) [1-2]. Through these examples of applications, the advantages and limits of TDA will be presented. TDA is insensitive to the presence of dusts (contrary to scattering techniques), and leads to a fair size distribution of the sample generally based on the weight-average of the constituents. With very small injected volumes (nL), application to ultra-small nanoparticles (below 5 nm), straightforward implementation, the absence of calibration, no filtration of the sample, TDA is a method of choice for the size-characterization of solutes in health applications. However, the repeatability / reproducibility of TDA can be affected by solute adsorption due to the deformation of the elution profile (peak tailing). A novel approach based on a “plug in front” methodology will be presented to limit the impact of solute adsorption in TDA. This approach is very general and can be used for any solute and any capillary surface. Combined with a rational and adapted choice of the capillary coating, this approach should address most of the solute adsorption issues in TDA applications.

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Influence of Transversal DC-Electroosmotic Flows on Dispersion Properties and Separation Efficiency in Hydrodynamic Chromatography

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Hydrodynamic chromatography is a microfluidic separation technique that was first proposed in the 1970s. After more than 50 years, it remains an underutilized method for characterizing particles ranging from tens of nanometers to a few micrometers, such as polymer mixtures, DNA fragments, and silver/gold nanoparticles. The separation efficiency, defined as the minimal channel length or the minimum operating time required to obtain a unitary resolution between two species, is affected by the weak driving force and the large values of the dispersion bandwidth of the species as the eluent velocity increases. Besides, using low eluent velocity causes challenges in the injection and detection systems, as well as an augmented operation time and a low handled amount of the sample. For these reasons, increasing the eluent velocity without compromising the separation efficiency is mandatory to broaden the domain of application of hydrodynamic chromatography. In this presentation, we show how the interaction between four different shaped transversal flows generated by DC-electroosmosis and the axial pressure-driven flow can contain axial dispersion as the eluent velocity increases. All the cases investigated showed decreased values of axial dispersion due to the localization effects of finite-sized particles in the region with lower axial velocity gradients. Due to this effect, specific of finite-sized particles, eluent velocity can be increased 50 times with respect to the standard case with no band-broadening effect. Brenner's macro transport theory and a Lagrangian-stochastic approach have been used to quantify the dispersion properties of finite-sized particles and the separation efficiency. The comparison between the two different methods yields perfectly matching results.

Droplet microfluidic platform for extracellular vesicle isolation based on magnetic bead handling

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Extracellular vesicles (EVs) are double-layered phospholipid vesicles having nanometric size that are rapidly gaining in popularity as biomarkers of various diseases, acting as cargoes of valuable information from the cell of origin (1). Despite their value, their current use in clinical practice is still limited. Among the limiting factors, one of the most critical is their isolation. In fact, conventional approaches are characterized by low purity and throughput, or poor reproducibility (2). Here, we propose a droplet microfluidic platform developed for EV isolation by affinity capture with magnetic beads. This platform is capable of processing large sample volumes (2 mL) in a relatively short time (4.5 hours), with a considerable automation. Systematic comparison with commercial methods proofs that the platform leads to an improved EV capture efficiency of 2.5-fold. This is due to the fact that EVs and magnetic beads are co-encapsulated within the same droplet, which acts promoting their mixing (3). Then, after the in-droplet incubation, beads are extracted within the microfluidic system and then collected for EV analysis (see Figure, top).
At first, the platform has been validated from the microfluidics point of view: throughput, automation and magnetic beads handling have been investigated. The latter is optimized by both numerical simulation and systematic experiments. Then, the EV isolation capability has been performed by the most used techniques: confocal microscopy and flow-cytometry prove the presence of EVs captured on the beads, while Nanotracking analysis (NTA) and protein assays (BCA and Western Blot) allow defining a capture efficiency of 56% (see Figure, bottom). Finally, the miRNAs cargo has been quantified to verify the EV integrity.

The remarkable improvements in terms of sample volume (2 mL) and analysis throughput (400 μL/h), compare with monophasic microfluidic approaches indicate how droplet microfluidics represent a suitable technology for EV isolation especially in case of clinical applications (4), where a few mL of starting sample is considered.

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Study of the hydrodynamics of micrometric particles within droplets confined in microchannels for biochemical applications

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Microfluidics is the science and technology that manipulates small amounts of fluids (pL-nL ranges), through channels of ten to hundreds of micrometers in size. A branch of microfluidics, known as droplet microfluidics, is experiencing a great impact in many biological applications (1), providing the production of highly controlled emulsions of droplets of an aqueous phase dispersed in oil, mixed with surfactants. Due to the presence of the droplet/oil interface, recirculation zones and stagnation points within the droplet appear modified compare with monophasic condition. This has important implications for the internal mixing of liquids confined in droplets as deeply investigated in the literature (2). However, if instead of liquid-liquid mixing, droplets contain micrometric particles, commonly used in biochemistry for isolation purpose, the recirculation is less obvious. In fact, depending on their size and density compared with the surrounding media, these microparticles may exhibit different behaviors. For example, according to droplet size and speed, particles are confined in specific regions (3, 4). Despite these preliminary results, a systematic study of the heavy bead recirculation dependencies from droplet volume, flow rate, channel dimension and the related consequences on specific applications is still missing. Here, we present a methodical analysis of the accumulation of beads denser than the medium (about 2-fold), for different capillary numbers (between 3e-04 and 5e-03), droplet volumes (30nL-3uL), and microchannel size (diameter from 300um to 800um). We observed that, as the droplet length over channel width ratio (L/W) of the droplet gets smaller, the mixing is improved. Moreover, increasing the flow rate causes the beads to accumulate at the rear of the droplet (see Figure). Then, the improved mixing provided to micrometric particles confined in droplets study has been tested by enzymatic reactions and extracellular vesicle isolation.

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Young Scientists in Field Flow Fractionation – Mission, Vision and Values

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The variety and complexity of field-flow fractionation-based techniques can be a challenge for young researchers or scientists new in the field. The wide-spread network of FFF experts as well as publications offer a lot of opportunities to connect and learn, but can be difficult to navigate. A well-structured organization-like system will help to minimize the hurdles users experience when getting started with FFF.

Our group of highly motivated young scientists is forming a committee in agreement with the Steering Board for International Symposia on Field- and Flow-Based Separations (SB-FFF), with the aims to 1) increase the visibility of FFF world-wide, 2) expand the FFF network between scientists, users and interested people with potential involvement of industry users, 3) offer online activities on the fffseparation.net platform such as seminars or Q&A sessions, and 4) facilitate a straight-forward knowledge exchange for both FFF beginners and experts. Based on our values, we are working on the actualization of the FFF online platform, the improvement of its quality, the initiation of outreach activities, and the sharing of knowledge via various online and in-person activities and social media channels, to make content more easily accessible. We hope that this initiative will provide a solid platform for researchers in the field, to create a sense of community and belonging, and to encourage new, especially young people to stay in the field of FFF, and to discover its potential as a valuable technique in a myriad of different research directions.

Characterization of temperature and carrier fluid viscosity effect in Gravitational Field-Flow Fractionation

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Gravitational Field-Flow Fractionation (GrFFF) is an elution-based method for the separation of particles of dimensions ranging from few micrometres up to about 50 μm of diameter. Particles are separated over time according to their size and other physical-chemical properties. Larger ones reach the end of the channel faster than the smaller, which move at slower velocities. It exploits the gravitational force acting perpendicularly in a laminar flow inside a ribbon-like thin channel. The fluid has a parabolic flow velocity profile across the thickness of the channel with the fastest flow velocity at the center and zero velocity at the walls. The retention time of the particles within the channel depends on their equilibrium position from the bottom wall. In this study, the effect of the viscosity of the carrier fluid on the elution behaviour (Retention time and Resolution) of polystyrene-based (PS) microparticles having dimensions of 7, 8 and 10 μm was investigated. First, it was studied how temperature modulates separation, through different carrier viscosity. Therefore, fractionation experiments were conducted at two different carrier temperatures: 14◦C and 28◦C. It was observed that for all PS microparticles at low temperature there is a decrease in retention time and a worsening of resolution, while at higher temperature the opposite effects have been obtained. To confirm that viscosity was responsible for this phenomenon, experiments were conducted at 28°C using a carrier containing 0.2% methylcellulose (MC), having the same carrier viscosity at 14◦C. It was observed that in the presence of methylcellulose, Retention times increase, and Resolution improves with a different effect depending on the size of PS microparticles, suggesting the presence of other phenomena acting on the separation. In conclusion, by adding MC to the carrier, it was possible to increase the resolving power of GrFFF by separating PS microparticles with only 2 μm difference, pushing GrFFF to new limits and opening up new possible applications. These studies conducted on PS beads are preliminary to future use on cells, as they have the same cell density and size, and this will allow improvement in cell separation for liquid biopsies with future applications in diagnostics.

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Join the community of researchers and scientists who rely on Shimadzu and TOSOH for their A4F polymer analysis needs. Together, we are shaping the future of polymer characterization with accuracy, reliability, and excellence.

KEYNOTE LECTURE 3

Field flow fractionation for the analysis of nanoplastics in relation to food safety

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Nanoplastics can be released from materials in contact with food and can infiltrate the food chain via polluted water, air, and soil. The scarcity of exposure data for nanoplastics in food hinders risk assessment and is mainly due to the lack of suitable analytical methods. Analyzing nanoplastics in food and biological matrices presents significant challenges because of their chemical nature, their small sizes, and expectedly low concentrations. While multidetector field flow fractionation (FFF) has been widely utilized for analyzing metal and metal oxide nanoparticles in recent decades, its application to nanoplastics within complex matrices is only just emerging. When paired with online detectors and offline analysis, multidetector FFF forms a robust platform for in-depth characterization of nanoplastics. This method aids in simplifying sample complexity, which typically obstructs the efficacy of most analytical techniques. The objective of this presentation is to outline the status of multidetector FFF in nanoplastics analysis, showcase examples of successful applications, and discuss future trends and requirements for advancing the analysis of nanoplastics.

Session 8: Food

AF4's contribution to the design of polysaccharide-based ingredients for food applications

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Polysaccharides from plants or algae are widely used as food ingredients. Starch is employed over the world for its functional properties in industry (gelation, stabilization, thickening, etc...) and for nutrition (energy source). Polysaccharide-surfactant blends, involving k-carrageenan for eg., are used in dairy, meat and bakery products, to stabilize emulsions and foams. Polysaccharides owe much of their functionality to the structuration of their assemblies, their size and the characteristics of their constitutive polymers. However, these structure-property relationships are not fully understood, probably due to method availability. A better knowledge of these parameters is therefore of paramount importance for the development of new food applications. Our approach is based on the determination of the structural heterogeneity of these biopolymers and their assemblies, in particular by analyzing their complete size distributions. As starch polymers, k-carrageenan, and their assemblies are not well fractionated by means of classical size exclusion chromatography (SEC), their structure and size are investigated using asymmetrical flow field flow fractionation (AF4) coupled with multi-angle laser light scattering (MALLS).

Through three examples, this talk will present the contribution of AF4-MALLS analysis to understanding: (i) the effects of k-carrageenan-anionic surfactant interactions on the properties of carrageenan-based gels, and (ii) the bread-making capacity and the digestibility of starch.

The macromolecular characteristics, supramolecular conformation and shape, determined through AF4-MALLS, SEC-MALLS, HPAEC-PAD (High Performance Anion Exchange Chromatography coupled with Pulsed Amperometric Detection), as well as microscopy techniques, will be discussed in relation to starch digestibility by amylase, and structure and rheological properties of the doughs and the gels. The information obtained helps to understand the behavior of these polysaccharide assemblies and opens new perspectives for designing new food ingredients.

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Differentiation of monovarietal wines from 4 autochthonous grape varieties combining sensory (Flash Profile) and analytical (AF4) studies.

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White wines contain macromolecules such as proteins, phenolic compounds and polysaccharides. These compounds participate in the textural profile or 'mouthfeel' that characterize the great white wines worldwide. Among these, the oily mouthfeel of wines from Viognier cultivar or the creamy texture that defines Chardonnay wines are perfect examples.

French Southwest Region has a real singularity compared to other wine regions with nearly 300 planted grape varieties of which 120 are autochthonous. The aim of this work was to select white wines from 4 typical varieties (Colombard, Gros Manseng, Mauzac and Far from the eye) from the South-West region of France with different taste characteristics and then to study their macromolecular structures using Asymmetrical Flow Field-Flow Fractionation (AF4). For this purpose, 69 wines were collected. They were analyzed by Fourier Transform Infrared Spectroscopy (FTIR) to determine the conventional enological parameters (alcohol content, titratable acidity, pH...).

After statistical processing by hierarchical ascending classification, 3 wines were selected by variety. A sensory test called "flash profile" was set up to differentiate the 12 according to their specific taste, thanks to an expert panel. Wines that stood out from the other wines on account of their characteristics were analysed in AF4.

The results showed that during tasting, two grape varieties stood out for their bitter flavor. In addition, when analyzed in AF4, the two varieties had different macromolecular profiles. The putative composition in proteins, polysaccharides, and polyphenols differed strongly between Mauzac and Colombard.

In order to study the impact of proteins, polyphenols and polysaccharides on the taste perceptions of the wines from Colombard and Mauzac, they were ultrafiltered at three different cut-off thresholds. The three collected fractions and initial wine are aimed to be tasted by the expert panel to determine the impact of macromolecules on the wines gustative properties, particularly their bitterness.

Exploring the power of colloidal fingerprints in the study of food matrices. A bovine milk case study.

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Food analysis is vital for consumer safety, authenticity assessment, fraud and pollutant detection. Yet the use of the traditional extensive and complex protocols often involving sample extraction and organic solvents call for more sustainable methods still able to deliver reliable, quick, and high-quality results. A recent trend in analytical chemistry involves the use raw data obtained from the analytical experiments (ex. chromatographic profiles and spectra) as fingerprints for a specific sample. The analysis of such data with multivariate tools allow to develop models able to predict samples characteristics (ex. geographical origin, sample composition) and identify adulterations. These approaches minimize the number of analyses, the laboratory work, sample and reagent amount therefore resulting in greener and more economic alternatives to traditional methods.
The application of such metrologies to data stemming directly from FFF analysis is still largely unexplored.

To advance this field we focused on bovine milk, an aqueous colloidal system of proteins and fat micelles that undergoes multiple processing (ex. thermal treatment, skimming) before being sold.

We developed a method based on a AF4-DAD-MALS-dRI-FLD platform able to separate the colloidal components of milk (serum proteins and aggregates, caseins ad fat micelles) and characterize them by the means of spectroscopy, laser scattering and FFF theory. The analysis of the sample fractograms with multivariate methods (PCA, LDA, PLS-DA) allowed multiple discriminations within a single run, such as thermal treatment and fat content; for the first time, also the impact of the manufacturing plant of milk on the colloidal content could be identified.

The developed AF4 separation method is fast, requires small amounts of sample and minimal sample preparation, with no chemical treatment and in saline conditions resulting in a greener and native method compared to common ones. The proposed approach can represent a starting point to improve the overall processes of quality control and fraud identification in the food industry and can easily be translated to other food samples presenting a colloidal content. Additionally, these results highlight the necessity of increasing the implementation of multivariate analysis to data directly provided by FFF platforms.

The delicate analysis of interacting proteins and their assemblies by flow field flow fractionation techniques

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Asymmetrical Flow Field-Flow Fractionation (AF4) is a separation technique that uses mild conditions, which are essential to investigate supramolecular assemblies. Here, we study the efficiency of several AF4 techniques to investigate the self-associating wheat gluten proteins. We compare the use of a buffer including 0.1% sodium dodecyl sulfate (SDS) and water/ethanol as eluent, on a model gluten sample. Thanks to a thorough analysis of dynamic light scattering auto-correlation functions measured in line, coupled with multi-angle light scattering (MALS) data, and the identification of molecular composition of the eluted protein by size exclusion chromatography in denaturing conditions, we evidence co-elution events in several conditions. We show that the initial focus step used in conventional AF4 with the SDS buffer leads to co-elution of protein monomers and aggregates, especially at short time. The frit-inlet device enables to decrease this coelution, but fractionation remains perturbated, probably because of the formation of charged protein-surfactant complexes. By contrast, an effective fractionation is obtained in water/ethanol using a small volume of injected sample. Loose assemblies of about 80 nm, with a molar mass of about 3.106 g/mol, enriched in glutenin polymers and ω-gliadin, are isolated at long elution times. Interestingly, despite the co-elution problems, the same protein composition is measured at long elution time in the SDS buffer. It demonstrates that the very large objects classically evidenced during AF4 analysis in SDS buffer are not high molecular weight glutenin polymers, as previously claimed by most studies, but complex assemblies interacting by weak forces, involving both monomeric and polymeric wheat gluten proteins.

Session 9: Polymers - part 2

Comparison of Size-Resolved Analysis of Dissolved Trace Elements in Soil Solutions Collected from Three Types of Lysimeters

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The distribution of dissolved trace elements (TEs) < 0.45 μm amongst predominant colloidal structures in soil solutions holds significance for their accessibility, availability, and potential toxicity to both plants and animals. These colloidal structures include labile complexes with simple inorganic ligands (e.g., Zn(OH)2, ZnSO4 (aq)), organic complexes and metal-bearing organic compounds (e.g., humic substances, porphyrins), and suspended inorganic solids (e.g., hydroxides, oxides). Asymmetric flow field-flow fractionation coupled to inductively coupled plasma mass spectrometry (AF4-ICPMS) separates dissolved colloidal structures based on their size, and analyzes associated concentrations of TEs. However, soil solutions collected using lysimeters tend to be limited to small sample sizes (10–20 mL). The optimal usage of small samples in multiple analyses combined with a shorter analysis time suggests the use of AF4 microchannel; however, these results have not been compared to the standard channel for environmental samples. Additionally, lysimeters only contain colloids associated with TEs below their pore size, leading to the collection of limited and potentially different colloidal fractions of TEs for various lysimeters. This presentation will discuss and compare the first size-resolved analysis of dissolved TEs in soil solutions using AF4-ICPMS with a 300 Da AF4 membrane in the microchannel and the normal channel, for three different lysimeters with different pore sizes, collected from three different soils. Lysimeter samples were filtered using 0.45 μm filters to obtain the dissolved fraction. The carrier fluid in AF4-ICPMS was adjusted to the pH and ionic strength of the sample to preserve the natural distribution of TEs. Soil solutions were also digested and analyzed in the ICP-MS to determine the overall TE concentrations, to assess the relevance of the dissolved fraction. Interactions between the soil solution and the lysimeter material may change the distribution of dissolved TEs among different inorganic and organic colloids in the sample. Nylon lysimeters, in particular, have the smallest pore size (0.2 μm inner), which will remove larger dissolved colloids associated with TEs, potentially underestimating the mobility of TEs.

Employment of ELSD with Linearized Signal for Sensitive Concentration Measurements in AF4-MALS

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Asymmetric flow field-flow fractionation with multiangle light scattering (AF4-MALS) is often used to separate macromolecules according to their size and to measure molecular weight distributions and molecular architecture. AF4-MALS is particularly useful in the characterization of branched and crosslinked macromolecules as well as for the presence of micro-gels, as it avoids anomalous late elution and permits separation of large sizes in comparison to size exclusion chromatography (SEC). In most cases, refractive index detector (RID) is used to measure concentration. The successful use of RID for concentration measurement can be particularly challenging for the low dn/dc materials especially when the concentration of the analytes components of interest is also low. Evaporative light scattering detector with signal linearization (HT-ELSD) from Agilent Technologies, Inc. have been employed to provide a sensitive alternative to RID for concentration measurements in high temperature SEC. In this presentation, we will be reporting the results of using this ELSD with room temperature AF4-MALS experiments for the analyses of industrial polymer samples. In contrast to RID, using ELSD allowed to detect the low concentrations of potentially relevant problematic constituents of the samples, thereby enabling determination of their structure and amounts to establish their role in the performance of the products. In addition, AF4 separations and concentration detection was possible to be carried out at lower overall sample loads avoiding the over-loading the AF4 channel. The presentation will show the comparison of results of the analyses with the ELSD and RID.

Studying Emulsion Polymerization Using Asymmetric Flow FFF in Various Modes

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Emulsion polymerization of acrylates and methacrylates provides waterborne latexes that can be directly used to formulate the final products such as paints or adhesives. The latexes are often characterized by the particle size distribution, mostly using batch dynamic light scattering. More detailed information about the particle size distribution can be obtained by coupling asymmetric flow field flow fractionation (AF4) with a multi-angle light scattering (MALS). Combining AF4 with the additional electric field offers the possibility to study the electrical surface properties of latex particles. However, the application properties of final products are controlled by the molecular structure rather than the size of the latex particles. Two crucial structural characteristics are molar mass distribution and degree of branching.

Emulsion acrylic copolymers often contain branched ultra-high molar mass fractions created by the chain transfer to acrylic segments of polymer chains. The proper characterization of such products by size exclusion chromatography (SEC) is practically impossible as the method degrades significantly the ultra-high molar mass fractions by shear forces. In addition, the large, branched macromolecules are prone to abnormal non-steric separation due to the anchoring in the pores of column packing. The recent trend of replacing petroleum-based monomers with renewable plant-based resources applies also to the chemistry of waterborne acrylic latexes. The traditional monomers can be partly replaced with vegetable oil-based monomers synthesized by the reaction of epoxidized fatty acid methyl esters with acrylic acid. As vegetable oils usually contain a certain fraction of polyunsaturated fatty acids, the bio-monomers partly crosslink the latex particles, which upon dissolution form nanogels, i.e., the entire swollen latex particles. The SEC analysis of such solutions is impossible as the nanogels are completely retained by the SEC columns.

For the complex characterization of acrylic latexes, AF4-MALS can be performed using an aqueous buffer to determine the particle size distribution and tetrahydrofuran to study the molecular structure with the MALS detector completed with an online viscometer. The obtainable information is: (i) particle size distribution, (ii) zeta potential with electrical AF4, (iii) molar mass distribution, (iv) branching, (v) content of nano gels in bio-synthetic latexes, and (vi) nano gel compactness.

Characterization of Ag-NPs hetero-aggregates formed upon interaction with mixture of natural macromolecules: insights from combined analysis using Classical and Frit-Inlet AF4

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The formation of biomolecular corona around nanoparticles is known to affect their fate and thus ultimate impact in environmental systems. The use of asymmetrical flow field flow fractionation multi-detection system (AF4-MD) is wildly applied to probe-out the formation (or not) of the biocorona at the surface of NPs, in the presence of individual proteins or in serum. More complex in composition, biomolecules released by microorganisms in freshwater environments consist of diverse mixtures of organic acids, and extracellular polymeric substances (EPS: proteins, polysaccharides...), polydisperse in size and with various reactivity. The present study aimed thus to explore how EPS released from *Cyclotella meneghiniana* (representative of freshwater diatom) may affect the elution of citrate stabilized silver-based NPs ((cit)AgNPs)) within AF4 channel, in order to probe out eco-corona formation. *C. meneghiniana* was grown for several days before EPS released in the medium were isolated by enrichment on ultrafiltration devices (> 1kDa). When dispersed in EPS solution, the elution of Ag-NPs was promoted in the AF4 channel with high recoveries > 75%, although (cit)AgNPs elution was completely abolished in their absence. This first suggested the formation of eco-corona around AgNPs. An increase in the retention of (EPS)AgNPs in the AF4 channel was however observed together with a decrease in recoveries when EPS quantities were decreased. Changes in retention behavior can be due to membrane conditioning by biocomponents which inhibit membrane fouling by AgNPs, and can affect the location of NPs during their relaxation, or due to in-channel aggregation. In-line Uv-visible scans and batch dynamic light scattering analysis indicated a decrease in agglomeration state of AgNPs associated to retarded elution, and that "in-channel aggregates" do not form. Using Frit-Inlet-AF4, known to decrease interactions with the membrane, (EPS)AgNPs were also eluted and with similar deviation from hydrodynamic elution. This excluded thus membrane-conditioning effect due to EPS, but corona-formation. Other characteristics of the aggregates formed (e.g. structure) are under investigation to explain deviations of the hydrodynamic elution. Our study validates the potential use of AF4 for the isolation of AgNPs hetero-aggregates driven by EPS from *C. meneghiniana*, rendering feasible the identification of eco-corona components by off-line techniques.

Characterization of titanium dioxide nanoparticles using asymmetric flow FFF hyphenated with multi-angle light scattering (AF4-MALS) and single particle ICPMS in environmental water samples

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The growth in use of engineered nanoparticles (ENPs) such as titanium dioxide (nTiO2) in consumer products is contributing to an increase in their release to the environment. Characterization of ENPs at environmentally-relevant concentrations in aquatic systems is particularly complex due to the presence of relatively large amounts of background mineral and biomolecular colloids of natural origin, and/or due to background substances released from nanoenabled products with the ENPs. Furthermore, in natural waters, the tendency of ENPs to homo- and heteroaggregate contributes to significant polydispersity which contributes to challenges in characterizing their size distributions. Knowledge of the ENP size-fractions is particularly important for risk assessments and understanding of ENP uptake and toxicity processes in organisms. In this study, we used AF4-MALS and spICPMS to characterize commercial nTiO2 used in paints and coatings, nTiO2 and developed protocols to optimize the range of size distributions that can be measured with reliable concentrations and size detection limits. AF4-MALS required at least 1 ppm mass concentration of nTiO2 for reliable size detection (20 nm in diameter). spICPMS had size detection limits of 20 to 25 nm for nTiO2 using the most abundant isoptope 48Ti (73.72%). The results of this study will contribute to the development of methods using novel techniques together to characterize ENPs in environmental compartments such as lakes and input streams such as runoff for better environmental risk assessment.

Session 10: Diagnostic - part 2

Development and harmonisation of robust and efficient fractionation strategies for characterisation of nanomaterials in biological matrices

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Nanotherapeutics has the potential to aid in responding to societal and medical challenges related to aging populations and an increase in chronic diseases. With a large number of new products being approved, there are pressing regulatory and industrial needs for preclinical characterisation of innovative nanotherapeutics. Development and validation of fit-for-purpose traceable methods are required to measure their stability, surface properties and biotransformation in biological fluids. Moreover, well characterised representative testing materials should be produced to enable method development and measurement quality control. The MetrINo European project, financed by the European Partnership on Metrology (EURAMET), spearheads the advancement of nanomedicine metrology. MetrINo supports industrial stakeholders in implementing regulatory directives, supporting the quality control of nanotherapeutics, including synthetic lipid-based particles such as liposomes and LNPs-RNA, and metal oxide nanoparticles (MONPs).

The general aim of the work here presented is to develop, validate and harmonise robust and efficient fractionation strategies, including Multi Detector Asymmetrical Flow-Field Flow Fractionation (MD-AF4) and Size Exclusion Chromatography (SEC) that are commonly used to separate nanomaterials from free proteins in blood plasma and other liquid biological matrices. The development of specific approaches for different classes of materials including liposomes, LNPs and MONPs will be presented. Method development is performed in two steps. First, the methods are developed for different classes of nanomedecines by playing on the separative parameters proper to each separation technique (channel/column, mobile phase, flows, speed, etc.). Then the fractionation approaches are applied and tested against selected liquid matrices containing serum proteins spiked with nanomaterials at clinically relevant concentrations. For AF4 and SEC fractionation methods, special attention is paid to particle recovery rates that should be ≥ 70 %, and repeatability/reproducibility of methods. For AF4, the targeted relative standard uncertainty should not exceed 5 % for the measured size, as recommended in ISO/TS 21362:2018 and CEN/TS 17273:2018. Results obtained by using standard analytic channel and frit-inlet configurations will be shown. A special focus will be on the transferability of developed protocols on different instrumental configurations.

Developed methodologies and validated methods are expected to provide the knowledge to feed urgent ongoing documentary standards.

Electrical-AF4-UV-vis-ICP-MS potential for the study of metallic nanoparticles in complex biological media

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Gold (AuNPs) and platinum (PtNPs) nanoparticles are being studied for biomedical applications due to their unique and interesting properties (1). When the NPs are dispersed in this biological media, they can suffer different transformations, such as aggregation/agglomeration, dissolution or even adsorption of different macromolecules as proteins. These processes modify the NP properties affecting their behaviour, fate, and toxicological profile (2). To understand these processes, analytical methodologies that allow isolation, quantification, and multicharacterization of NPs in complex matrices (as biological media) are needed, but they are still in an early stage of development.

Among the available techniques, electrical asymmetrical flow field-flow fractionation (EAF4) has emerged as a promising option for metallic NPs. It can be coupled online to multiple detectors, such as ultraviolet/visible absorption (UV–vis), fluorescence emission or inductively coupled plasma mass spectrometry (ICP-MS), which give different but complementary information for the characterization of the NPs including electrical parameters (electrophoretic mobility and zeta-potential) (3). However, it is still underexploited, and the capabilities should be demonstrated. Thus, the aim of this work has been to develop and examine a new analytical strategy via EAF4-UV-vis-ICP-MS for the study of the AuNP and PtNP behaviour in complex biological media, such as bovine serum albumin, fetal bovine serum (FBS), and cell culture media (Dulbecco's Modified Eagle Medium, DMEM). The presence of both NPs in these media resulted in an increase in their hydrodynamic diameter. Also, the media composition had a significant effect onto zeta-potential and electrophoretic mobility of the NPs. Furthermore, in the specific case of AuNPs, an oxidation process was noted when they were dispersed in FBS and DMEM. This work demonstrates the potential of EAF4-UV-vis-ICP-MS for study changes of the physical-chemical properties of AuNPs and PtNPs while they are dispersed in complex biological matrices and will be further applied.

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Biophysical investigation of immunostimulatory complexes of LL-37 with nucleic acids

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Autoimmune diseases and inflammatory disorders have a growing prevalence in our aging population, and the antimicrobial peptide LL-37-part of the first-line defense of the human innate immune system in fighting pathogens-appears to be significantly involved. LL-37 has been shown to possess antiviral, antifungal, and antibacterial properties and is beneficial for human health in this way (1). Due to its structure and composition, it is affine to forming complexes with other biomolecules.

LL-37 in complexation with different kinds of nucleic acids has been shown to be immunomodulatory and appears to be involved (at least) in psoriasis and other autoimmune diseases in unclear ways. On the other hand, these complexes have antimicrobial effects, beneficial for human health. (e.g. (2)) We hypothesize that dysregulation of LL-37 in the human body and the resulting complex conformation might control whether complexes show beneficial health effects or are implicated in deleterious inflammation and in the development of autoimmune diseases (see (3)).

It is apparent that the analysis of complexation patterns of LL-37 with different nucleic acids will be an essential step to elucidating LL-37's role in the human innate immune system. To our knowledge, no systematic molecular-scale studies have yet shed light on the dynamics of formation and structural details of these physiologically important complexes of LL-37 with nucleic acids. A combination of Size Exclusion Chromatography (SEC) and Asymmetric Flow Field-Flow Fractionation (AF4) in combination with different detectors offers a straightforward approach to revealing the macromolecular structures of the complexes. In new work presented here, we use SEC to analyze peptide assembly and complexation of up to 150.000 g/mol whereas AF4 allows us to investigate larger molar mass aggregates. The combination of separation science with Multi-Angle Light Scattering enables us to gain information on size, conformation, and aggregation stages. Complexes of LL-37 and nucleic acids elute in the void peak of AF4 making extensive method optimization necessary. Further, we employ a variety of analytical approaches, including microscopic and scattering techniques.

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A fast, low-volume and high-throughput HF5 approach to investigate biologically active extracellular vesicles from plasma of patients with Polycythemia Vera

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Extracellular vehicles (EVs) are bilayer membrane nanoparticles with a high potential in determining cancer diagnosis and prognosis. Isolating and characterizing EVs from plasma is challenging due to low concentration, high heterogeneity, and nanoscale contaminants. Current isolation strategies (ultracentrifugation, size-exclusion chromatography (SEC), ultrafiltration) usually require large sample amounts (hundreds of μL) and suffer from low efficiency in analysis time and purity. Additionally, they risk compromising analyte integrity and biological activity. We present an approach able to isolate biologically active EVs from low amount of plasma (60 μL per subject) exploiting a Hollow-Fiber Flow Field-Flow Fractionation Multidetection (HF5-multidetection) platform. Our miniaturized device allowed for a fast (< 25min) separation and simultaneous spectroscopic size and shape characterization of the analytes by online detectors (DAD, MALS, FLD) while providing high throughput, soft and native separation, and minimal dilution of the separated fractions collected at the end of the platform. Performance comparison with a traditional method (SEC cartridges) showed improvement in terms of analysis time, sample amount, dilution and characterization as well as in terms of preservation of vesicular structure and biological activity. The described platform was exploited to separate and analyse plasma samples from both healthy donors and patients affected by Polycythemia Vera (PV), a clonal disorder of hemopoietic stem cells. EV-enriched fractions resulting from HF5 separation were collected and characterized by offline techniques which showed successful purification from plasma protein, biological activity and EVs marker expression.

Overall, these results suggest that HF5- multidection platform can be an important asset in unravelling the role of EVs in orchestrating the complex interplay between PV tumour cells and the inflammatory and senescent microenvironment, that is still poorly understood.

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Development of an AF4 analytical approach to understand the complexity and evolution of cell secretomes, and to identify the effect of purification techniques

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Background

More refined and complete characterization of cell secretomes are needed to accelerate the development of extracellular vesicles (EV) derived medicine towards clinical applications. The AF4 separation technique (Asymmetric Flow Field-Flow Fractionation) is well suited to fractionate complex media into subpopulations, in order to characterize each of their individual components. It can be used for quality control, or to identify potent fractions. Currently, most EV studies focus on exosome isolation with little interest in the other elements produced by the cells. AF4 can be developed in a dedicated fashion to address EV issues. The goal of this work is to establish a polyvalent separative method to use AF4 at its full potential for extended secretome characterization.

Material & Methods

Conditioned supernatants recovered from 2D HeLa-cultures placed in complete medium or serum-deprived medium for 48h were used to model 2 types of secretomes. They served to fine-tune the AF4 separative and detection parameters. Isolated subpopulations were identified using inline AF4 detectors (MALS/DLS/UV-Vis/dRI), dot blotting and cryoEM. The purification of HeLa conditioned media was performed by ultracentrifugation (UC), tangential flow filtration (TFF) and ultrafiltration (UF) and analysed by AF4.

Results

The AF4 separation method developed allows to track the evolution of a cell secretome composition over time. The coupling with dot blotting and electronic microscopy is a powerful tool to get access to the identity of each subpopulation. The comparison of three EV purification techniques by AF4 shows that UF and TFF can conserve the full sample with little changes regarding subpopulation proportions while increasing concentration. UC selects a range of sizes depending on the centrifugation parameters and can isolate subpopulations.

Conclusion

AF4 coupled to MALS/DLS/UV-Vis/dRI analyses is effective to investigate fine variations in biological samples, spot effects related to production and isolation protocols and identify the composition of a mix. This new approach paves the way for further multimodal analyses, and the use of AF4 as a quality control in the field of nanotherapeutics and bioproduction.

SdFFF as a label-free technology for cell separation and personalized medicine in oncology: application in colorectal cancer.

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Over the last decade, sedimentation flow/force fractionation technology has emerged as a promising approach for cell separation in oncology, specifically targeting cancer stem cells (CSCs) in colorectal cancer and glioblastoma. These CSCs are crucial due to their involvement in cancer recurrence. However, current sedimentation field-flow fractionation (SdFFF) methods lack the capability to identify CSCs during isolation.
This project aimed to overcome this limitation by developing a coupling between SdFFF and a biosensor tailored for CSC identification. The ultimate goal was to automate CSC separation and characterization. To validate this coupling, several biological identifications of CSCs sorted by SdFFF were conducted.

Additionally, to assess CSC properties and determine optimal chemotherapy treatments, 3D models of Multicellular Tumor Spheroids (MCTS) derived from SdFFF-isolated CSCs are under development. The objective is to conduct chemotherapy tests and potentially establish this approach as a personalized medicine method for future colorectal cancer patients.

To enable the coupling of SdFFF with the Biosensor, the phosphate-buffered saline (PBS) mobile phase was replaced with dielectrophoresis medium (DEP) necessary for biosensor usage. New elution conditions for DEP were identified, and biological characterizations of post-SdFFF sorted cells were conducted, including proliferation assays, cell cycle analysis, RT-qPCR, and dot blot proteomic analysis. to obtain deeper insights into the genomic characteristics of the sorted fractions, which will enhance understanding of the correlations between the biosensor signatures and the biological states of the fractions.

Biological characterization revealed CSC enrichment in the F1 fraction for RNA expression and the F3 fraction for functional properties. We observed that cells recovered in F3 post-SdFFF had a significantly lower signature than in other fractions and were comparable with cells' signature when cultivated in defined medium, indicating enrichment in CSCs.

In conclusion, this study highlights the potential of SdFFF with the Biosensor to automate the identification and isolation of colorectal cancer CSCs. This coupling facilitate the creation of 3D models from patients for chemotherapy sensitivity testing in the future.

Session 11: Standardization

Bayesian evaluation of nanoparticle size distribution: from batch MALS to multidetector-AF4 metrologically integrated approach

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Nanoparticles samples of a given chemical compound generally present an arbitrary particle size distribution. The size distribution, and not only the average size, is the first rank characteristic of performance and toxicology of nanomedicines. Many ensemble-method measuring instruments reduce to estimation of the average size. Algorithms have however been developed for computation of a distribution, but none present a concept of uncertainty on the distribution itself. Uncertainty is one of the cornerstones of metrology and a gap is thus currently present. With an implementation for several instruments and together with an evaluation of their uncertainties, the distributions can then be compared with each other, or combined for hybrid metrology applications where the strengths and weaknesses compensate among the different techniques.

Uncertainty evaluation and comparability between measurement techniques are the purpose of the work presented here. It is first realized by implementing a Bayesian inversion of the size distribution of batch Multi-Angle Light Scattering (MALS) measurements, together with uncertainty on it. We show that sensitivity of the instrument naturally arises from the uncertainty evaluation.

In a second step, the retention time of nanoparticle sample separation by Asymmetric Flow Field- Flow Fractionation AF4(-UV) is exploited to upgrade the model by introducing a Bayes prior obtained from retention time. Gain in sensitivity is illustrated for small nanoparticles (below 30 nm) by considering AF4(-UV)-MALS in the same paradigm. Alternatively, outlook of considering AF4-UV size measurement by retention time, per se, is considered. Comparison to other measurement techniques and especially Dynamic Light Scattering (DLS) , which rely on the same theoretical measurand as AF4 – the coefficient of diffusion – can bring insight into sample properties, chemical stability and surface properties, and is at the core of multidetector-AF4. These advances could, in turn, help in better understanding and subsequently in improving the theoretical description of AF4 separation dynamics.

Inter-comparison of Different Fractionation and Batch Methods for the Physicochemical Characterization and Quantification of Nanoplastics

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Nanoplastics (synthetic polymer particles < 1 μm) are abundant pollutants and of great ecotoxicological concern. Thus, they are an ongoing topic of research. To ensure comparability between studies on nanoplastics, it is essential to perform inter-comparison of various methods. Here, the feasibility of separation/fractionation methods such as field-flow fractionation (FFF)– multi-angle light scattering (MALS) and centrifugal liquid sedimentation (CLS) for analysis of nanoplastic size, shape, and quantification of particles was evaluated in comparison with batch methods, including dynamic light scattering (DLS), nanoparticle tracking analysis (NTA), tunable resistive pulse sensing (TRPS). Additionally, the potential of FFF techniques (asymmetrical flow FFF and centrifugal FFF) combined with Raman microspectroscopy (RM) or pyrolysis gas chromatography mass spectrometry (pyGC-MS) for the chemical characterization of nanoplastics was studied. For inter-comparison of different methods, a set of representative/test particles, including polydisperse polyethylene (PE), (doped) polystyrene (PS) nanoplastics, titanium dioxide, and iron oxide nanoparticles (spherical and elongated), was used. This complex set was chosen to move a step closer toward real nanoplastics and to assess the applicability and limitations of the selected methodologies for this analytical challenge. While results on size and particle number concentration of orthogonal batch methods (DLS, NTA, TRPS) were comparable for monodisperse spherical samples, higher deviations were observed for polydisperse, agglomerated samples and for non-spherical particles. FFF-MALS showed good recoveries for mono- and polydisperse samples (including agglomerates) of different materials. Furthermore, preceding separation allows for better size characterization of complex samples (agglomerated or polydisperse). FFF in combination with RM (online) or pyGC-MS (offline) provide complementary data on physical and chemical properties.

The online-coupled FFF-RM approach can deliver size-resolved chemical information down to 100 nm, while offline coupling to pyGC-MS allows for identification of polymers and for mass-based quantification. While combined methods can deliver an extensive data set within one measurement, batch methods may still be required for specific detailed information (morphology by TEM/SEM or high-resolution size characterization by CLS). Furthermore, significant improvements in sample preparation, including preconcentration and/or enrichment steps are required to overcome seri- ous challenges with respect to the identification, characterization, and quantification of nanoplas- tics using the evaluated techniques.

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Standards for the analysis of environmental samples using AF4-ICPMS

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Asymmetric flow field-flow fractionation (AF4) coupled to inductively coupled plasma mass spectrometry (ICP-MS) has a range of applications in environmental nanogeochemistry, particularly to analyze different size fractions that correspond to different forms in environmental samples, e.g. small molecules and simple complexes < 300 Da, DOM-associated forms, small inorganic forms and larger inorganic forms. The size of these colloids is directly related to their mobility, reactivity, and potential toxicity. However, stable colloidal standards suited to the development and verification of AF4-ICPMS to analyze complex environmental samples are still to be developed. Thus, this presentation will discuss a reproducible standard composed of an organic and inorganic colloid mixture with associated trace elements for the analysis of environmental samples using AF4-ICPMS. Organo-mineral iron(III) oxyhydroxide (FeOx) colloids were formed by oxidation of Fe(II) in the presence of different concentrations of humic acids. The molar DOC/Fe ratio in test solutions was varied to determine the optimal ratio for stability of the standard and reproducibility of the procedure. The viability of a mixed DOM-Fe(III)-trace element colloid standard based on its stability over time will be discussed, along with the reproducibility of its manufacturing process as determined by analysis using AF4-ICPMS. Colloid mixtures were filtered to obtain the < 0.45 μm fraction. Temperature, pH, and ionic strength were measured and controlled to maintain reproducibility. Analysis was also compared for AF4 using the conventional and microchannel, for the first time using a 300 Da membrane in the latter. The results of interlaboratory comparisons using the standard may also be discussed.

POSTERS

Nanoparticles impact on human health: development of a nano-bio interaction model

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Since the early 2000s, nanoparticles usage flourished in day-to-day life, in various fields such as cleaning supplies fabrication or biomedical diagnosis for example. One consequence is that nanoparticles and their by-products can be released in the environment, especially in wastewater. Effects of nanoparticles contaminated water on fauna and flora are mostly unknown and in need of further investigation. First scientific results on nanoparticles-cells interactions, also known as nano-bio interactions, suggest an impact on human health.

In biological research, several biological models have been developed over the years to become more and more representative of what could be going on inside of an individual. Starting in the early 1900's with monolayered cell cultures in two-dimensions (2D), researchers are now able to produce complex systems ranging from three-dimensions (3D) culture to organ-on-chips and animal models. The general consensus at the moment is that 3D models, organized in a similar manner than *in-vivo,* are more suited for interaction analyses than 2D cell cultures because of changes in metabolism and signalization.

To analyze and characterize interactions between nanoparticles and human cells, we've decided to produce a 3D biological model called a spheroid. First, human cancer cells are sorted according to their bio-physical characteristics by a Sedimentation Field-Flow Fractionation method (SdFFF). A sub-population enriched in "Cancer Stem-Cells" (CSCs) is obtained and cultivated into a spheroid. Because of their self-renewal abilities and tumorigenic capacities, using CSCs instead of an heterogenous population will further increase the chances of getting a standardized and reproducible 3D model.

Then, several concentrations of silica nanoparticles are applied onto cancer cells and the interaction is monitored over time. The goal of this thesis is to optimize the model developed previously, and use it to monitor nano – bio interactions.

In this presentation, model development strategy will be presented and first results concerning nanoparticles and biological model get-together will be discussed. By using several technics of brightfield and fluorescent microscopy, we're going to take a look at size and structure of spheroid models, as well as viability, cytotoxicity and cell death mechanisms during nano-bio interactions.

Towards a workflow for comprehensive analysis of nanoplastics

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Nanoplastics, defined as plastic nanoparticles smaller than 1 μm, are known as a ubiquitous environmental pollutant. However, analysis of nanoplastics present in the environment poses several serious issues, such a versatility of particle sizes, polymer identities, surface modifications and extremely low mass concentrations. Therefore, nanoplastics analysis is currently still in its infancy (Mitrano et al., 2021).

Asymmetric Flow Field-Flow Fractionation with Multi-Angle Light Scattering detection (AF4- MALS) has been suggested as a tool for size characterization of nanoplastics (El Hadri et al., 2021). However, AF4-MALS does not provide information on particle chemistry, which is a considerable hurdle taking into account great complexity of organic particles naturally present in the environment. Pyrolysis-gas chromatography-mass spectrometry (Py-GC/MS) has long been used for identification and mass quantification of polymers. Therefore, we investigate the possibility of combining AF4-MALS with Py-GC/MS in one workflow.

The preliminary experiments carried out on polymers and polymeric standard nanoparticles suggest that direct coupling of AF4-MALS with Py-GC/MS, though less time-consuming, might not be efficient enough for analysis of environmental nanoplastics because of the high detection limits. Additionally, Py-GC/MS poses certain requirements for the samples, such as lack of involatile inorganic salts, which has to be taken into account during AF4-MALS method development. We therefore propose a workflow involving large-volume AF4-MALS injection (10 mL of sample), size separation in volatile carrier liquid, additional concentration step and quantitative sample preparation for Py-GC/MS. In this way, AF4-MALS serves as both sample-cleanup and size characterization, while Py-GC/MS is a detector ensuring polymeric nature of the analyte.

Top-down analysis of exosome lipids by mAF4-ESI-MS/MS

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Exosomes, extracellular vesicles in the size range of 30-150 nm, play a crucial role in intercellular communication and are expected to serve as useful biomarkers for various diseases. The conventional lipidomic analysis of exosome lipids is typically performed through bottom- up analysis by liquid chromatography-tandem mass spectrometry (LC-ESI-MS/MS), qualitatively and quantitatively analyzing the total lipids after extracting lipidome of biological origins with organic solvents. This study aims to demonstrate the potential of top-down analysis by miniaturized asymmetrical flow field-flow fractionation and electrospray ionization-tandem mass spectrometry (mAF4-ESI-MS/MS) as a high-speed screening platform for direct analysis of exosome from blood sample without extraction. Using an mAF4 channel, exosomes from serum sample can be separated at high speeds (< 15 min) with an effluent flow rate of up to a few tens of microliters per minute and fed directly into ESI-MS/MS for the screening of target lipid species. For the comparison, bottom-up analysis was carried out for intrahepatic and extrahepatic cholangiocarcinoma serum samples for selection of lipid biomarker candidates, and as a result, 25 lipid species were selected as biomarker candidates for top-down analysis. Targeted quantification by top-down analysis is expected to demonstrate statistical agreement with the quantified results obtained by bottom-up analysis. In the context of exosome lipid analysis, this study contributed to demonstrating the potential of mAF4-ESI-MS/MS as an effective high-speed screening platform for top-down analysis.

High throughput Asymmetrical Flow FFF for purification of proteins

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Protein purification is a critical and expensive step in the production of pharmaceuticals. Current protein purification systems are highly advanced chromatographic techniques, such as affinity, ion exchange, and size exclusion chromatography, each tailored to exploit specific properties of the target product. For a large-scale operation, the cost of purification can be a few hundred thousand dollars, with resin materials costing a few thousand dollars per liter of product. These figures underscore the immense financial stakes in protein purification within the pharmaceutical industry, driving the continuous search for more cost-efficient and innovative purification technologies.

It has been shown that incorporating a scaled-up channel allows the Postnova Asymmetrical Flow FFF (AF4) to be utilized as a semi-prep scale separation technique for the nanoparticles (1). This study presents an assessment of new Postnova semi-preparative scale AF4 channels for protein purification. The high throughput AF4 system boasts the process capability to handle 3 mg of protein per injection, marking a tenfold increase compared to the capacity of the analytical system. With continuous operation around the clock, the system is capable of processing up to 1.7 grams of protein each month. Moreover, the operational expenses are significantly low, only amounting to $70 per membrane in addition to the cost of buffer usage. Additionally, the system's efficiency is highlighted by the minimal downtime required for column regeneration and validation, which typically ranges from days to weeks but is now reduced to merely a few hours.

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Gold Nanorods as Immunogold Labels: Purification via Asymmetric Field Flow Field Fractionation

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In cancer research and clinical cancer diagnosis, immunolabelling techniques are crucial. They are used to localise and ideally also quantify the expression of antigens that can predict disease progression and treatment outcomes. Immunogold labelling is a technique in which small gold nanoparticles are used as contrast agents (labels). The ultrastructure of cancer cells and their particles can be directly visualised through electron microscopy (EM). By means of Tokuyasu processing and cryo sectioning, the ultrastructure is well preserved.

Immunolabelling commonly uses small gold nanoparticles (AuNP) with diameters below 20 nm. For multiplexed immunolabelling, it is necessary to use different batches of particles that can be unambiguously distinguished by size, shape, and/or contrast in electron microscopy. Small gold nanorods (AuNR) are an excellent addition to the portfolio of suitable labels due to their distinguishable shape from AuNP. When staining two targets simultaneously, AuNP and AuNR are intentionally used as two labels with different shapes. Therefore, it is essential to remove AuNP formed as a side product during AuNR synthesis to avoid misinterpretation of AuNP from different origins, i.e. intentionally used AuNP labels vs. AuNP introduced by unpurified AuNR.

Asymmetric Field Flow Field Fractionation (A4F) is an excellent separation tool for purifying and characterising AuNRs on-line using Dynamic Light Scattering (DLS) and UV/Vis extinction spectroscopy. A4F effectively separates AuNRs and AuNPs of similar sizes based on their different diffusion coefficients, allowing for elution at different retention times.

This study presents immunogold labelling results on Tokuyasu cell sections using AuNRs purified by A4F. AuNR purified by A4F can be confidently used for immunolabelling, as demonstrated by the successful simultaneous staining of two cancer-related targets on SkBr3 breast cancer cells. This highlights their potential to enhance the current immunolabel portfolio, which is so far primarily composed of spherical AuNP. A4F plays a key role for obtaining purified gold nanoparticles with different shapes.

Characterization of nucleic acids using Asymmetric-Flow Field-Flow Fractionation (AF4)

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Asymmetric-flow field-flow fractionation (AF4) is a gentle separation method that applies liquid flows to separate sample components based on their hydrodynamic sizes (Parot et al., 2020). After the use of Covid-19 mRNA vaccines with their outstanding example of global benefit during the pandemia (Watson et al., 2022), this separation approach equipped with detectors has been extensively used to characterize the physicochemical properties of RNA-lipid nanoparticles (LNPs) (Bian et al., 2023).
To result in a successful nanoparticle formulation and finally in efficient cells transfection, such nanotherapeutics rely on high-quality nucleic acid molecules (Ouranidis et al., 2022). Therefore, there is a demand for methods that enable analysis of the quality of RNA before incorporating them with lipids to form RNA-LNPs.

In this study, we present case examples of using AF4 to analyze integrity and purity of mRNA. Comparing different RNA purification methods and examining the effect of mRNA forced degradation by temperature, we demonstrate AF4's ability to spot the presence of unwanted byproducts and RNA monomers. In addition, we compare AF4 to existing methods analyzing nucleic acids such as Capillary Gel Electrophoresis (CGE) and fluorescence-based kits.

In cases mentioned above, AF4 fractograms provide valuable information on the quality of the studied mRNA molecules, where produced and purified nucleic acid samples are separated from the contaminating reaction components. In addition, measuring quantity of RNA is possible by adding a calibration curve of a reference RNA material in the sequence. Besides including a pre-run mobile phase to establish RNase-free conditions in the AF4 environment, no complicated measures are needed making this method relatively fast and easy.

Application of asymmetric flow field-flow fractionation (AF4) for the characterisation of CellInject, a potential innovative antimalarial drug delivery system

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Malaria is an infectious disease that remains a global health burden, necessitating innovative antimalarial drug delivery strategies to improve efficacy and sustainability, with a specific focus on controlled drug release mechanism. This study explores the viability of CellInject®, a lipid-based nanoparticle to be used as an antimalarial drug delivery system to deliver the poorly soluble active antimalarial drug to infected cells.

For the optimal development of this antimalarial drug delivery system, and to ensure the desired particle properties are obtained, multidetector asymmetrical flow field-flow fractionation (MD-AF4) was employed as an advanced characterisation technique. Additionally, complementary characterisation techniques were also employed to evaluate the particle size, surface charge, determine the particle number concentration and to conduct initial investigation into particle and drug release profile. With the aid of MD-AF4, the particle size, polydispersity, and particle shape of various formulations of the CellInject® lipid-based nanoparticles as well as CellInject® lipid-based particles encapsulated with various antimalarial drugs, were evaluated.

The particles, irrespective of being unloaded or encapsulated with antimalarial drug, were found to be narrowly dispersed and to have a diameter of ±40 nm. By using MD-AF4, it was possible to determine that the particles are ellipsoidal in shape. The particles were investigated under different simulated physiological conditions and were found to be stable, as no significant change in its physical properties was observed. With the aid of nanoparticle tracking analysis (NTA), it was possible to determine both the hydrodynamic size and the absolute particle number concentration, which assisted in the initial investigation into understanding the release profile of these particles. The combination of techniques provided insights into the physicochemical characterisation of the complex CellInject® drug delivery system which is necessary for the optimisation of such a drug delivery system.

Investigating the potential of thermal flow field-flow fractionation in antimalarial drug delivery system characterisation

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Thermal field-flow fractionation (ThFFF) has been proven to be a powerful and versatile separation technique, which has been utilized for the characterisation of nanoparticles. The ability of ThFFF to separate complex samples based on normal diffusion and thermal diffusion, make it an useful tool in the characterisation of antimalarial drug delivery systems.

This study aims to explore the potential of ThFFF for the characterisation of antimalarial drug delivery systems in aqueous media. In this regard, CellInject®, a lipid-based nanoparticle to be used as an antimalarial drug delivery system is characterised. These lipid-based drug delivery systems are considered to be complex, due to the intrinsically inherent multiple molecular distributions (e.g. the possible formation of micelles concurrent with the lipid-based nanoparticles). Moreover, the quantitative and qualitative analysis of the antimalarial drug associated with the drug delivery system contributes to its complexity. Given ThFFF's high resolution capabilities, the objective is to optimize the separation and (1) comprehensively identify the components present in the specific formulation of the drug delivery system; and (2) obtain information on the size and size distribution of the drug delivery system. This will enhance our understanding of the composition and particle interaction within the drug delivery system.

In addition, the objective is also to investigate the potential application of ThFFF for the quantification of the amount of free drug present within a given formulation. Complementary technique such as DLS and NTA were also used to obtain further valuable insights into the physical properties of the lipid-based drug delivery system.

Influence of different membrane coating on the measurability of pDNA-polyplexes in the asymmetrical flow field-flow fractionation

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The steadily rising interest in the investigation of interactions between nanomaterials and biofluids has led to an increasing interest in asymmetrical flow field-flow fractionation (AF-FFF). Compared to other analysis techniques, the strength of AF-FFF is the possibility to alter the flow profiles, eluent composition and membrane material to suit a specific separation problem. A particular field of interest is the investigation of the protein corona formation on nanoparticles when they come into contact with biological media. This is especially relevant for particulate systems that are envisaged to be used as drug delivery systems. Potential candidates are plasmid DNA (pDNA) polyplexes, i.e., electrostatic complexes of negatively charged pDNA with cationic polymeric systems. In this work, we investigated if and under which conditions different types of pDNA polyplexes can be fractionated in an AF-FFF channel. For this, a number of measurements using different membrane coatings was carried out using pDNA polyplexes with different surface modifications and the influence on the fractograms was evaluated.

Separation and characterization of extracellular vesicle with asymmetrical flow field-flow fractionation

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Extracellular vesicles (EVs) are vesicular structures that are produced by cells for intercellular and intracellular communication. To fulfil different needs, EVs are selectively loaded with functional molecules such as lipids, proteins, metabolites or genetic material. Depending on the carried information and on the origin of the EVs, these have a characteristic range of particle/structure size. Depending on their size, EVs are categorized in subclasses: Exosomes are of endosomal origin and usually have a size in the range of 50-150 nm; microvesicles are formed by the cellular plasma membrane and their sizes are in the range of 150-500 nm; oncosomes are send out by cancer cells and are atypically large with sizes in the range of 0.5-10 μm. By monitoring these biomarkers, diseases could be identified at an early stage and the progress of a medical therapy could be observed. It has been shown that the fractionation of EVs within an asymmetrical flow field-flow fractionation (AF-FFF) channel is possible, meaning that AF-FFF is a suitable analysis technique for the characterization of EVs. Compared to other methods such as ultracentrifugation and size exclusion chromatography, the fractionation with AF-FFF is less time consuming and offers the possibility of an online analysis e.g. by light scattering for size determination. Due to the lack of a stationary phase, the risk of disintegration of the EVs is low and AF-FFF has a high recovery rate, which allows following analyses. Within the work presented here, EVs from cell culture are investigated and a method is established to separate the larger EVs (microvesicles, oncosomes) from the exosomes. The independent analysis of the different EV subclasses aims to support the development of anti-cancer therapy approaches by providing the possibility of a tight monitoring of the cell communication.

Development of an assymetrical-flow field-flow fractionation methodology for the chracaterization of α-Galactosidase a loaded-nanoliposomes

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α-galactosidase A (GLA) enzyme replacement therapy emerged as a golden standard in treating Fabry disease. However, its limited efficacy in patients, short half-life and high cost present some drawbacks that can be overcome by coupling enzymes to drug delivery systems. As an alternative to conventional therapeutic approaches, nanoliposomes constitute an attractive carrier platform for the targeted delivery of therapeutic biomolecules, such as GLA (1–3). The characterization of such delivery platforms is the key requirement for the identification of critical quality attributes (CQAs), in particular, particle size distribution, shape, morphology, drug encapsulation, and stability (4). In this study, we have developed and optimized a method based on asymmetrical-flow field-flow fractionation coupled with multi-angle light scattering and refractive index detectors (AF4-MALS-dRI). The results showed the efficiency of the developed method to provide valuable insights into the size, shape and physical stability of the nanoliposome structures. Moreover, a comprehensive analysis of factors influencing the fractionation process and subsequent physical characterization was conducted to ensure the best possible separation and to explore a wide range of CQAs. Once the method for empty nanoliposomes was established, GLA-loaded nanoliposomes were also examined using the same method. Encapsulated nanoliposomes were slightly larger and showed broader peak in comparison to empty ones. Therefore, the developed methodology proved to be useful in investigating size and morphology differences between liposomal nanoformulations. Although this method requires additional parameters to be investigated, it encourages the development of drug delivery nanosystems and might be adapted for other lipid-based nanotherapeutics with some modifications.

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Asymmetric Flow Field Flow Fractionation (AF4) for Characterization of Polymersomes from Block Copolymers of Different Architectures

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Polymersomes are artificial vesicles formed by self-assembly of amphiphilic block copolymers in an aqueous medium. Polymer membranes are typically thicker, more rigid, more stable and less permeable than those made of lipids. The improved stability of polymersomes makes them attractive for numerous applications in drug delivery, biomedical diagnostics, nanoreactors, nanosensors and cellular model systems. Many of these applications require improved selective membrane permeability, which can be achieved by incorporating protein biopores. The incorporation of biopores into polymer membranes is often hindered by excessive membrane thickness, insufficient membrane stability or misorientation of the proteins after insertion. Therefore, there is great interest in the development of novel polymer membranes that would allow a successful incorporation of proteins and the formation of biomimetic polymersomes. Here we study the effects of different architectures of block copolymers (AB linear, ABA linear, and AB2 miktoarm star) on the size, morphology, membrane thickness and stability of polymersomes. The polymersomes were prepared by the thin-film hydration method followed by extrusion. Polymersomes were then separated by size using AF4 coupled to the multidetector system, consisting of a multi-angle light scattering (MALS) photometer with an embedded dynamic light scattering (DLS) module, a refractive index (RI) detector and a UV detector. Using this approach, we determined the average size and size distribution (hydrodynamic radius, Rh, and radius of gyration, Rg), as well as the particle shape. AF4-MALS was used for stability studies, while cryo-EM imaging allowed us to visualize the vesicles and determine their membrane thickness.

Realistic Nanoplastics and their Detection using Field-Flow Fractionation

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Plastic pollution is a worldwide problem; and whilst the larger plastic pollution items can cause an issue with endangering animals, it is a lesser discussed issue that could cause the most impact and pose a bigger threat to the animals (and to humans). Nanoplastics are tiny plastic particles measuring less than 1000 nm that are a potentially larger environmental concern of plastic pollution with profound implications. Some papers suggest that nanoplastics have the ability to cross cell membranes due to their small diameters. This area of research is, however, still in its early stages, with much nanoplastics research being dominated by polystyrene nanoparticles made using bottom-up methods which produce monodisperse nanoparticles that are spherical in shape and have smooth surfaces. Our research will address this by producing realistic nanoplastics using top-down methods.

Nanoplastics are colloidal particles and so are likely to aggregate, especially in complex media as present in the natural environment. The aggregation of nanoplastics, whether heteroaggregation with other organic colloidal matter, or homoaggregation, will play a significant role on how the nanoplastics behave. Currently, there is limited knowledge regarding the colloidal stability of nanoplastics in the environment, an issue that our research investigates. Due to their small size of nanoplastics, they are difficult to identify and analyse as most instruments do not have the resolution, additionally the presence of other organic colloidal matter in samples can make it very challenging to characterise nanoplastics within a complex media. To tackle this issue by using a field flow fractionation technique, namely asymmetric flow field-flow fractionation. The ability to separate the nanoplastics from the rest of the organic matter will provide valuable insights into the issue of nanoplastic pollution, as well as information crucial to forming effective methods to mitigate their future impacts on the environment.

This work will explore the production of producing realistic nanoplastics using mechanical abrasion techniques how their exposure to environmental media (such as pond or sea water) affects their colloidal properties. This analysis is enabled using asymmetric flow field flow fractionation to separate and characterise the particles and determine the factors that influence aggregation behaviour.

Understanding Nanoplastics' Fate in the Human Digestive Tract by AF4 and light scattering techniques

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The surge in plastic pollution has raised concerns about the presence of nanoscaled plastic particles (nanoplastics) in food. While animal studies highlight the hazardous impact of selected nanoparticles (Yong et al., 2020), the behavior of nanoplastics in the human digestive tract, particularly the fate between ingestion and absorption to human cells, remains a significant knowledge gap.

Our research focuses on assessing the fate of well-defined polystyrene nanoparticles in the human digestive tract. We systematically evaluated the effects of size, surface modifications, and morphology. Simulating the stomach and small intestine conditions through two phases of *in vitro* incubation, we mimicked parameters such as temperature, acidity, shaking, and the presence of specific enzymes, revealing a tendency of nanoparticles to agglomerate.

Characterization of samples was conducted using asymmetrical-flow field flow fractionation (AF4) with UV and multi-angle light scattering (MALS) detection. Complemented by dynamic light scattering (DLS) and scanning electron microscopy (SEM), our study offers a comprehensive overview of nanoparticle changes within the gastrointestinal system. Preliminary conclusions shed light on the potential absorption of nanoplastics via oral exposure, urging further exploration of associated human health risks.

Molecular characterization of infernan polysaccharide grafted with a thermoresponsive polymer by FI-AF4-MALS-dRI

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Infernan, an anionic slightly sulfated exopolysaccharide (EPS) secreted by *Alteromonas infernus*, a bacterium from deep-sea hydrothermal vents, displays some biological properties similar to glycosaminoglycans of mammalian tissues. For its use in cartilage tissue engineering as thermoresponsive and injectable hydrogel, infernan was covalently grafted with poly(N-isopropylacrylamide) (pNIPAM) via carbodiimide chemistry. Even if several studies have reported on anionic polysaccharides grafted with pNIPAM, only a few of them determined the molecular characteristics of grafted polysaccharide chains, including their molecular weight and conformation as well as the degree of grafted uronic acid, due to the complexity of long polysaccharide chains. Thus, this work aimed to fully characterize the physicochemical characteristics of infernan grafted with pNIPAM using Frit Inlet-Asymmetrical Flow Field-Flow Fractionation coupled with Multi-Angle Light Scattering and Refractive Index detectors (FI-AF4-MALS-dRI). By varying EPS/pNIPAM molar ratio, four EPS-pNIPAM were obtained. They differed by their EPS ratio (9% to 33% w/w) and their degree of grafted uronic acid (2% to 16%). An important increase in weight-average molecular weight, Mw, was observed for grafted EPS (Mw > 109 g/mol) together with a five-fold increase in radius of gyration, Rg (500 nm), when compared to starting infernan displaying Mw of 1.6x106 g/mol and Rg of 100 nm. In addition, the hydrodynamic coefficient indicated a change in molecular conformation upon grafting. Indeed, a random coil conformation was observed for pure EPS (0.73), while a net decrease in the hydrodynamic coefficient by one order of magnitude was noticed for EPS grafted by pNIPAM samples (between 0.02 and 0.06), indicating a dense spherical structure in solution. It appeared that changes in physicochemical parameters of EPS-pNIPAM samples resulted from inter-chain associations between polysaccharide chains mediated by grafted pNIPAM, which led to the formation of large aggregates.

In conclusion, FI-AF4-MALS-dRI analysis allowed a better understanding of the relationship between the structure of infernan grafted by a thermosensitive polymer and the functional properties of the resulting EPS hydrogels. This knowledge remains essential for future use of thermosensitive infernan-based hydrogels in cartilage tissue engineering.

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Using asymmetric flow field-flow fractionation hyphenated with multiple detectors for the analysis of pharmaceutical bionanomaterials

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Biotechnology is a rapidly growing industry, encompassing a diverse molecular landscape and providing new medicines for areas of unmet clinical need. A critical aspect in the development of novel bionanotherapeutics is developing a robust understanding of their physio-chemical properties, which dictate quality, safety and efficacy. Flow field-flow fractionation has emerged as such a technique, offering versatile solutions for the separation and analysis of biotherapeutics under formulation and biologically relevant conditions, which can be implemented for the early evaluation of preclinical formulations. We present the characterisation of an IgE formulation following exposure to formulation (pH) and environmental stress (thermal and freeze-thaw) by heating either to 80◦C for 15 minutes and 56◦C for 24 hours. We investigated the impact of stress on the aggregation status using AF4 -UV-Vis, and multi-angle light scattering (MALS), using size exclusion chromatography and orthogonal sizing techniques. We show the loss of monomeric purity of IgE following thermal stress, which was absent for samples subjected to freeze-thaw stress indicating adequate formulation stability in response to freeze-thaw stress. Analysis by dynamic light scattering (DLS) and nanoparticle tracking analysis (NTA), show a similarly significant change in the emergence of aggregation at elevated temperatures. Overall, we show that AF4 can be used as an orthogonal gentle separation technique to perform the high-resolution analysis of antibody stability during early formulation screening.

Would You Like a Carrier With That?

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Intro: Ribonucleic acid therapies are currently in the spotlight of nanomedicine field with FDA approval of several therapies, three of which utilize lipid nanoparticle drug delivery carrier systems (1). With the growing popularity of a novel therapeutic platform, subsequent analytical methods transferred from nanomedicine analytics have been adopted as routine, gold standard techniques which have not faced similar growth and development.

LNPs have the potential to revolutionize the drug delivery field, however as a vast array of excipients can be used within the formulation of LNPs, a lack of deep analytical profiling during early development stages, can delay the translation time of these therapies to the clinic. Here, we use a model Poly(A) and cationic lipid nanoparticle system, complexed with Poly(A), and highlight differences in critical quality attributes measured at different manufacture steps, and demonstrate the need for Field-Flow Fractionation high resolution instrumentation (2).

Methods: Poly(A)-DOTAP-LNPs were manufactured *via* microfluidics using the lipid composition 1,2-dioleoyl-3-trimethylammonium-propane:cholesterol: 1,2-distearoyl-sn-glycero-3-phosphocholine: 1,2-dimyristoyl-rac-glycero-3-methoxypolyethylene glycol-2000 (DOTAP:CHOL:DSPC:DMG-PEG2000) at a molar percentage ratio of 50:38.5:10:1.5. and purified. Poly(A) and LNPs were analyzed using dynamic light scattering (DLS), and nanoparticle tracking analysis (NTA). Both sample types were measured using developed AF4 methods for evaluation beyond the scope of DLS/NTA.

Results: LNPs were predominantly in the 60 nm size range, with a low polydispersity/size distribution (> 0.2), near neutral surface charged (> +10 mV), and high drug loading (> 95%). Further FFF analysis demonstrates changes in drug morphology following complexation with a model carrier system.

Conclusion: Overall, we have demonstrated the impact of carrier system utilization on Poly(A) through evaluation of both Poly(A) drug and LNP system critical quality attributes through their evaluation of low and high-resolution analytical techniques.

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Speciation of Ru colloids through AF4-MALS

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Objectives: Ruthenium is considered to be an interesting element for establishing a 103Ru/103mRh generator for Auger therapy. In radiochemical processes, Ru may be present as Ru(II), (III), (IV), (VI), (VII), or (VIII), with Ru (III) and Ru (IV) the most numerous and stable compounds (1, 2). But a chloride solution initially contains both Ru(III) and Ru(IV) species (1). Ru species chemical behaviors are strongly pH dependent, leading to polynuclear complexes. This tendency to form polynuclear complexes linked by oxide and hydroxide bounds is most prominent for the oxidation states +III and +IV (3). Additionally, colloidal ruthenium hydroxides are formed by the hydrolysis of Ru(III) and Ru(IV) compounds. These polynuclear, polymeric and colloidal species are not desirable for establishing a well-controlled chromatographic 103Ru/103mRh generator.

Methods: Asymmetrical Flow-Field- Flow Fractionation (AF4) has been be used to analyze different Ru(IV) solutions in different HCl concentrations to check the presence / absence of Ru colloids in order to establish suitable HCl conditions for elution, i.e. keeping monomeric Ru(IV) on the resin when avoiding Ru colloids formation. The speciation of Ru was carried out by Asymmetrical Flow Field-Flow Fractionation (AF4) coupled to a Multi-Angle Light Scattering (MALS) detector.

Results: The presence of ruthenium colloid in aqueous solution was evidenced with a polymodal and polydisperse profile. Ruthenium colloids formation appears before a precipitation within RuO2, visible at pH 2 and pH 4. Ruthenium colloids observed in the supernatant seems to be more stable at pH 4 than at pH 2 since no colloid has been observed at pH 2 either by MALS or by UV. These data lead to assume that it is possible to make generator in acidic pH conditions because in this case, all the species are in solution, and no colloids are formed

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Analytical possibilities in the combination of AF4 and Nanoparticle Tracking Analysis (NTA)

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Pharmaceutical nano-formulations may contain a plurality of colloidal materials. A technique such as dynamic light scattering (DLS; very popular and essentially the standard in the field) suffers of intrinsic limitations, the most important of which is its skewed sensitivity to objects with higher scattering (e.g. those with a larger size, that do not swell, with loads of heavy heteroatoms etc); it also cannot differentiate objects on the basis of chemical composition. It goes without saying that the use of DLS as a detector after fractionation (e.g. with AF4) makes it in principle possible to analyze complex colloidal mixtures. Here we show, however, that the use of both DLS and NTA (as AF4 detectors, in combination with refractive index detection) allows for a more quantitative appreciation of the composition of colloidal mixtures; here we report the case of mixtures of differently sized (60, 125, 250 nm) polystyrene latexes (Figure 1). We further show that the use of NTA in fluorescence allows the identification of poorly scattering materials also when mixed with more scattering ones; here, we show the analysis of fluorescently labelled hydrogel nanoparticles (poorly scattering due to their swelling by water) mixed with the latexes above (Figure 2). The take-home message is that the results obtained from the AF4-DLS combination should be better confirmed by other techniques, due to the questionable comparability of the scattering data from differently sized particles.

Polymeric Micelles via Polymerization-Induced Self-Assembly (PISA): Structural Analysis and Stability in Complex Biological Media

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Polymerization-induced self-assembly (PISA) has proven to be a versatile route towards high concentrations of micellar nanostructures with tunable chemistries and morphologies. In contrast to conventional self-assembly protocols, where the synthesis and assembly of the block copolymer (BCP) building blocks are performed in two separate and consecutive steps, PISA relies on a one-pot procedure where BCP formation and assembly occur simultaneously. Typically, solvophilic polymers carrying polymerization initiation sites are mixed with a chemically distinct monomer. Polymerization of this monomer yields BCPs with an amphiphilic character, triggering assembly into (higher-order) micellar constructs. The morphological details of these structures are time-dependent and are in principle tunable by the extent and kinetics of the polymerization.

Recently, the PISA concept was coined as an attractive route for the synthesis of drug delivery vehicles. Here, the simplicity of a one-pot procedure and the ability to perform PISA reactions at high solid contents can be combined with *in situ* encapsulation of (model) hydrophobic pharmaceutical compounds. Although appealing, the understanding of how the assembly pathway impacts the physical-chemical properties of the micellar nanostructures (such as their size and shape) and their stability in complex biological media is lacking.

In our study, we use a model system based on poly(ethylene glycol)-*b*-poly(diacetone acrylamide) to investigate differences in the stability of PISA-derived BCP micelles of different sizes. Their stability is assessed with two complementary strategies: (A) Monitoring of the particle size with dynamic light scattering (DLS) during incubation with a small surfactant and (B), biologically more relevant, through incubation with plasma. Given the complexity of the latter, we rely on the separative power of asymmetric flow field-flow fractionation (AF4) to dissect the influence of (individual) plasma components on the stability and degradation of the micelles. After further comparison with micelles prepared via conventional self-assembly protocols, we ultimately aim to formulate pathway-structure-performance relations towards highly efficient drug delivery micelles.

Asymmetrical flow field-flow fractionation-multidetector for the characterisation of metal oxide nanoparticles used in nanomedicine

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In the past two decades, numerous nanoparticles have been developed for different medical purposes like drug delivery, as a contrast agent or an imaging agent to diagnose and stage diseases (in oncology, neurology). This field named nanomedicine has gained a greater interest these past years, notably with the Covid vaccines. Nonetheless, there is a huge lack in available and representative standards and standardization procedure to assess the quality of these nanoparticles and to evaluate their potential side-effect related to the nanometric scale of these scaffolds. Thus, there is an unmet need claimed by the European Medical Agency for developing and standardising new methods related to the quality and safety assessment of nanomedicines. Among the parameters to be determined for characterizing the nanoparticles, their size and size distribution are of utmost importance since it could influence their biodistribution in vivo (with also the surface charge).

Among the size characterization techniques, asymmetrical flow field-flow fractionation (AF4) hyphenated with multi angle light scattering (MALS) offers the advantage of a smooth separative technique, without denaturation of the sample during its analysis, together with providing the size distribution of the sample.
In this work, the development of the AF4-MALS method for the characterisation of different metal oxides used as magnetic resonance imaging (MRI) contrast agents will be presented. The effect of critical parameters like the carrier solution, and the cross flow and other important elution parameters will be highlighted.

Multi-detector centrifugal FFF for the establishment of procedures for the development of synthetic and biological nanocarriers

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Magnetic Nanoparticles (MNP) serve as markers for biological and synthetic carriers used to investigate metabolic processes or develop cell therapeutic approaches. For this purpose, carriers such as lipid particles or cells are loaded with MNP to enable precise localization and tracking within the body by imaging methods such as magnetic particle imaging (MPI), an outstanding technique for quantitative MNP detection with exceptional temporal resolution and sensitivity. The greatest challenge in labeling cells and carriers with MNP is to achieve a sufficiently high loading while maintaining the functional properties of the carrier and the imaging performance of the MNP. The influence of the individual preparation steps and substances used in cell loading with MNP is largely unexplored, as traditional ensemble measurement methods have poor selectivity and specificity, especially when applied to mixed particle size populations. However, the aggregation of MNP in cell culture media can lead to a sensitive disturbance of their MPI imaging properties and therefore complicates the establishment of a targeted labeling method.

With the help of centrifugal field-flow fractionation coupled to a unique multi-detector platform consisting of UV-, dynamic and static light scattering, as well as Magnetic Particle Spectroscopy (MPS) for determining concentration, structure, and magnetism of MNP in cell culture medium, it has been possible to optimally coordinate MNP selection and medium composition and thus achieve reproducible cell labeling with previously unattainable magnetic signal properties. We present these results and show that in particular the use of the magnetic MPS detector is suitable for quantitative determination of the MNP-containing size population.

This measurement infrastructure represents a powerful and indispensable tool for the reliable and controlled development of MNP-labelled synthetic and biological carriers.

Miniaturized thickness-tapered channel in flow field-flow fractionation

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Flow field-flow fractionation (FlFFF) is an effective separation technique for the separation of samples based on particle size. The thickness-tapered channel in FlFFF was introduced recently to improve separation speed and efficiency as much as field programming which requires an additional flow controller. A comparison of FlFFF with field programming and the thickness-tapered channel demonstrated that FlFFF with a thickness-tapered channel could improve resolution and sample recovery simultaneously. Top-down analysis has become possible through the on-line coupling of FlFFF and electrospray ionization tandem mass spectrometry. Since mass spectrometry needs a relatively low flow rate, a miniaturized FlFFF channel was invented to maintain resolution and sample recovery at a lower flow rate. By miniaturizing the thickness-tapered channel for FlFFF-ESI-MS/MS, the improved separation efficiency of the thickness-tapered channel can be utilized for top-down analysis of biological particulate materials at a reduced outflow rate condition. In this study, FlFFF with the miniaturized thickness-tapered channel was tested for its separation performance by separating polystyrene standards of which sizes are similar to those of exosomes. This presentation will demonstrate the performance of a miniaturized thickness-tapered FlFFF channel for particle separation which can be in the future integrated with MS instrument.

Responsivity analysis of smart nanoparticles using Asymmetric Flow Field-Flow Fractionation

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Future research on advanced nanoparticles (NPs) for drug delivery systems (DDSs) will be based on smart and biodegradable polymers. Amphiphilic block copolymers can self-assemble into NPs, specifically polymersomes, which are versatile carriers due to the possibility to tune their properties at the molecular level and their potential to encapsulate a range of bioactive compounds (1). Smart polymersomes, in particular, exhibit a variety of characteristics that are essential for DDSs as they are stimuli-responsive and can modify their structure or morphology in response to a given stimulus. More complex structures have been developed to respond to the presence of multiple stimuli such as dual-responsive polymersomes (pH and temperature) which exhibit morphological changes depending on the characteristic pH value and lower critical solution temperature (LCST) of the corresponding polymers (2). A detailed understanding of self-assembly, responsive behaviour, and the influence of structural features are required to develop smart polymersomes for a given application. The separation and characterization of such complex self-assemblies prove to be a challenge due to their polydisperse nature and the possible decomposition under strong shear forces. Asymmetric flow field-flow fractionation (AF4) is an emerging technology for the detailed separation and characterization of complex, fragile self-assemblies (3). Coupled with a number of highly sensitive detectors, AF4 can deliver essential information about the molar mass, shape and conformation properties. Consequently, multi-detector AF4 may be used to characterize smart NPs and investigate their responsivity. (4)

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Advancements in Inorganic Ion Analysis: Improved Recovery and Separation Techniques in CyElFFF with Conductive Resin Filled Carbon Electrodes

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Inorganic ions are critical in biological, environmental, and industrial processes, emphasizing the importance of their effective analysis and isolation. Field Flow Fractionation (FFF) and its sub-method, Cyclical Electrical Field Flow Fractionation (CyElFFF), have emerged as promising techniques for the retention and separation of inorganic ions. However, challenges persist, especially in the low recovery and separation of inorganic ions in the presence of anions. This has led to the exploration of alternative electrode materials used in CyElFFF channels with superior surface properties (such as lower porosity) compared to conventional carbon graphite. Results from CyElFFF using Conductive Resin Filled Carbon Graphite (CRF CG) electrodes show a significant improvement in ion recovery, including up to 99% recovery without applied voltage and between 65% to 80% with applied voltage, which varies depending on the electric field. This method demonstrates a notable enhancement in cation-anion separation compared to previous models using channels of similar height (25.4 mm). However, challenges remain in anion-anion separation, particularly for Cl and NO, as the peaks for anions are generally wider and their retention times similar. The experiments indicate that the CRF CG channel offers the best separation resolution (Rs) for Na+ and K+, with an Rs of 1.3 at Vpp= 2 V and f= 0.5 Hz under the tested electric field conditions. For cation-anion separation, the highest Rs was 2.6 for KCl and 2 for NaCl at Vpp= 3 V and f= 0.5 Hz. Additionally, the best resolution between the first retained peak and the void peak exceeded 1.5 for KCl and reached 2 for NaCl at Vpp= 4 V and f= 2 Hz.

Membrane material screening for analyte-specific repulsion in asymmetrical flow field-flow fractionation

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For asymmetrical flow field-flow fractionation (AF-FFF) experiments, usually commercially available membranes are used that haven't been created for this use only but thrive commonly in industrial filtration applications. In AF-FFF, separation mechanism is influenced by possible interactions between sample and membrane. Analyte-specific changes in the membrane's characteristics should make measuring suitable for samples that were previously impossible or complicated to measure. In addition, the separation performance of the AF-FFF can be improved by using analyte-specific repulsive forces of the membrane. The commonly used membrane materials are polyethersulfone and cellulose, with pore sizes of 1 - 100 nm. Both of which have negative zeta potential in aqueous environment. Therefore, membranes with a positive zeta potential are desirable. To achieve such polymers that have a positive zeta potential, polymers with a high amount of Lewis-base were prepared. Polymers that are most suitable to these needs are, for example, high-nitrogen species like polyazoles or tertiary amines in general. Depending on the manufacturing process of choice, a range of porosities is generated. On a laboratory scale, precipitating the polymers from solution and squeegeeing them in order to create a thin film is a proven method. For industrial production, spraying polymers onto a higher-permeable carrier material or film extrusion are continuous processes to create membranes. Based on existing approaches, membranes of polyazol and polyimid derivatives were cast from a solution containing the water-soluble polyvinylpyrrolidone as the polymer to be washed out. As a comparative value, polyethersulfone was prepared in a similar fashion. The membranes were characterised and tested on the AF-FFF by using standardised particles with known zeta potential. In addition, REM imaging was used to qualitatively analyse the membrane surface for defects, uniformity, and pore size.

Utilizing deterministic lateral displacement (DLD) array to sort microbes in glacial meltwater

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The glacial archives are worth to be analyzed due to its isotope and micro-organism inside, in order to reconstruct and study the ancient environment. The genome of microbe in meltwater was analyzed since forty years ago. Due to the fact that some samples are not culturable, these samples should be analyzed immediately after the melting process of ice-core. Despite of the focus on the research in culturable microbe, there are still plenty of room for exploring and developing the method in culture-independent microbe analysis. Here we present a microfluidic technology called DLD array, which was emerging twenty years ago, to sort particles in terms of its sizes. The remarkable advantage of the method is its high resolution, some researches have demonstrated that it could separate particles in nanoscale. The ultimate target of our project is to separate bacteria, fungi, and cells which was included in glacial meltwater, and group them respectively on its size, so they can be further analyzed in genome and other biological aspects. By applying DLD microfluidics, the samples were gradually sorted from large to small in several steps. We have therefore proposed two DLD arrays, with two critical diameters. After a certain number of experiments, we hereby declare that the smallest sample with 1 and 3 um particles were successfully separated. This work aims to apply microfluidics in the research of glacial meltwater, which is still unusual, so it is possible to understand the correlation between the microbe sizes and its genom.

Characterization of ultra-small metallic nanoparticles by Size Distribution - Taylor Dispersion Analysis (SD-TDA)

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Formulation of nanoparticles, pharmaceutical analytes require a fine characterization of their size and stability across different parameters such as temperature, pH, and solvent nature, however the current techniques present difficulties in complex media, involve a high volume for analysis, a sensibility for sample's polydispersity, a large bias and sensitivity for aggregates and fluorescent samples (1). Herein we present an innovative, rapid and accurate analytical technique based on Taylor Dispersion Analysis (TDA) (2). This method which is based on the dispersion of solutes upon a laminar flow, requires a few nanoliters volume, and allow the determination of the diffusion coefficient interpreted as the hydrodynamic diameter via the Stokes-Einstein equation. In this study we describe the size determination by TDA of ultra-small gold and silver nanoparticles synthesized by reduction with trialkylsilane of metal salts dissolved in hexane. The size of the metal NP cores was determined by transmission electron microscopy (TEM) and small-angle X-ray scattering (SAXS) and the nature of the capping agent by X-ray photoelectron spectroscopy (XPS). The hydrodynamic diameters of 5 and 7 nm found by TDA for the Au and Ag NPs, respectively, is very consistent with the mean diameters of 1.8 nm (Au) and 3 nm (Ag), determined by TEM and SAXS and the presence of an oleylammonium chloride shell of thickness 1.5-2 nm. In addition to the size measurements, this work shows the powerful of the Taylorsizer platform, which can record nanoparticles and ligands UV spectra and follow the efficiency of the purification.

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Size Distribution by Taylor Dispersion Analysis (SD-TDA): An innovation for resolutive size distribution measurement of lipid nanoparticles and monoclonal antibodies

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The assessment of the absolute size of mixture of nano-objects is a challenge in biophysical characterization. There are a limited number of affordable technologies that provide accurate particles size distribution down to 0.5nm. Current technics such as DLS, A4F/SEC-MALS, NTA are often not suitable to work in native form. Indeed, denaturation occur during sample preparation or analysis: filtration, dilution or shear stress.
Nanoscale Metrix developed a new methodology to characterize the size distribution of nanoobjects, the Size Distribution Taylor Dispersion Analysis. With SD-TDA, only 10 nL of sample are mobilized under low pressure differential in a capillary. Under Taylor dispersion conditions, the hydrodynamic radius can be calculated from the detected signal (U.V, L.I.F) through Stokes- Einstein equation.

In this work, SD-TDA was first used as a new alternative method to determine the size and size distribution of nanovectors: LNPs encapsulating mRNA, coated metallic nanoparticles.

The size of LNP is known to affect intracellular delivery and therefore vaccine efficacy and SD-TDA allow a very accurate size measurement. Thus, several LNP formulations were successfully analyzed and obtained results were compared to those obtained with DLS or FFF-MALS. Second part of this work was dedicated to the thermal stress of human IgG. At high temperature, a kinetic study of the IgG degradation was carried out. A modification in the IgG size is measured and degradation products were also detected. The size of obtained IgG fragments is compared to the size of purified fragments directly characterized by SD-TDA (Fc, Fab, and F(ab')2 fragments)

High-throughput milk A2 β-Casein analysis by capillary zone electrophoresis (CZE)

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Cow milk contains 13 types of β-casein with A1, A2, and B being the most common ones. In some cases, consumption of milk containing A1 β-casein was associated with increased gastrointestinal inflammation and worsening of post-dairy digestive discomfort symptoms. These symptoms can be avoided by consuming milk containing only the A2 β-casein. These findings led to increased demand for cow milk with high A2 β-casein content. Only 30% of cows produce just A2 β-casein and these special cows need to be identified, segregated, and milked separately from the rest of the herd. Authentication of high A2 β-casein content in milk from the A2 herds is required to ensure the quality of A2 milk. In this poster, we describe an easy, fast, robust, and high-throughput CZE analysis method to determine the percentage of A2 β-casein in milk. It provides high-resolution separation of A2 β-casein from other β-casein proteins, with excellent repeatability and reproducibility for accurate and quantitative analysis.

Connecting isoelectric focusing to mass spectrometry using the IntaBio Imaged CIEF-MS System for charge variant analysis.

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The IntaBio System directly couples iCIEF charge variant analysis with high-resolution MS detection for direct in-line peak identification of intact proteins. The analysis of mAbs using the IntaBio System takes about 15 minutes per sample, much faster than the days or weeks required for traditional workflows such as IEX with fraction collection. In this poster we will describe how this system can be used to directly identify charge variants of adalimumab, a therapeutic antibody.

Flow Field-Flow Fractionation for Investigation of Nanoparticles in Consumer Products

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Nowadays, various consumer products in the market are prone to incorporate nano and microparticles to improve efficiency but mostly for increasing of products value and using as a selling point. Moreover, most vendors only provide the information of particles before including into their manufactured goods. However, they rarely follow and report the fate of the particles in their products. It is necessary to gather data of added nano or microparticles in products so that the safety information can be systematically studied and evaluated. Therefore, consumer products in Thailand were investigated by using Flow field-flow fractionation (Flow FFF) coupled with various detectors such as UV-Visible spectrometer, multiangle light scattering (MALS), and inductively coupled plasma mass spectrometer (ICP-MS). Furthermore, Fourier-transform infrared spectrometer (FTIR) and fluorescence microscope were applied to characterize released microparticles in consumer products. Various types of metal nanoparticles, nano-polymer, microplastics were obtained. The profiling of nano- and microparticles was considered to assess their capability in and toxicity in consumer products for further application.

Size Characterization of Wheat and Soy Proteins by Frit-Inlet Asymmetrical Flow Field-Flow Fractionation (Frit Inlet AF4) before and after High Moisture Extrusion-Cooking

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Frit Inlet AF4 coupled to Multi Angle Light Scaterring (MALS) and UV detectors has been investigated to better understand protein structuration during High Moisture Extrusion- Cooking (HMEC). Over the past decade, interest in the HMEC process has been growing as it makes it possible to transform various plant proteins into meat analogues revealing a fibrous texture. The structuration of the extrudates is still not fully understood, and varies according to the protein source. In this study two model raw materials have been studied: soy concentrate (66,43% protein) and wheat gluten (82,44% protein), for which protein solubility and molecular structure vary. It has been observed that increasing the amount of wheat gluten (from 25% to 75% (w/w)) led to more compact and oriented extrudate structure. The use of Frit Inlet AF4 enabled to characterize, without any protein modification, the raw material in two different solvents: ethanol/water (50/50 v/v) suitable for gluten proteins solubilization and solution of NaCl (0,5M) suitable for soy proteins solubilization. The same solubilization has been conducted on the extrudates. Diluted solutions have been analyzed by AF4 and Frit-Inlet AF4 for the fractionation of small and larger protein assemblies, respectively. The objective of this study was to compare the size of these assemblies before and after HMEC processing, and to compare the effect of an increasing amount of gluten on the size of free and non-reticulated protein aggregates in the extrudate. It shows the full potential of Frit Inlet-AF4-MALS-UV to analyze plant protein meat analogues and to help the understanding of phenomena involved during the process.

Understanding the bread-making potential of native and fermented amylose-free cassava starch as a new gluten-free ingredient through macromolecular characterization by AF4-MALLS

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Cassava is a staple food particularly in Africa. Moreover, it is the second most important source of starch in the world. Cassava starch offers numerous advantages as an ingredient: it is gluten-free, non-allergenic, has a neutral taste, a high level of purity, and excellent thickening characteristics. Moreover, a natural modification of cassava starch, involving anaerobic fermentation and ultraviolet light exposure through sun-drying, is traditionally used in South America, to produce sour starches with unique expansion properties. Recently, naturally occurring amylose-free cassava mutations have been reported. Native amylose-free starch is a cost-effective and eco-friendly alternative to current options in cassava. It also exhibits high expansion properties. The aim of this work was to assess its potential and the macromolecular determinants of its specific expansion properties for the development of gluten-free ingredients for clean-label baked expanded products. The bread-making potential of amylose-free and wild- type cassava starches fermented and oven or sun-dried was evaluated by measuring the specific volume after baking. The best results were typically achieved by the synergistic combination of fermentation and sun-drying. Native amylose-free starches had twice the bread-making capacity of fermented wild-types. To gain insight into the mechanism underlying this outstanding expansion performance, the macromolecular characteristics of three starches with contrasting expansion properties were determined using AF4-MALLS, and their evolution tracked during fermentation in relation to bread-making capacity. Other structural properties, such as crystallinity and chain length distribution were also monitored. Macromolecular information shows that the bread-making potential is mainly determined by the number of linear chains and is favored by fermentation and sun-drying through a reduction in amylopectin molar mass and the production of macromolecules with increased branching degree.

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Automating sample preparation for the characterization of biotherapeutics using CE-SDS, cIEF and Glycan analysis

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This poster describes the full automation of 3 key applications in the characterization of biopharmaceuticals using capillary electrophoresis: Capillary electrophoresis sodium dodecyl sulfate (CE-SDS)[1], capillary isoelectric focusing (cIEF)[2] and glycan (FG)[3] from sample preparation to CE separation. In general, the methods prepare the samples in a 96-well plate format, and all reagent plates are used to complete the electrophoretic analysis.

The automation platform used in this study was the Biomek i5 MC workstation from Beckman Coulter Life Sciences followed by sample separation using capillary electrophoresis technology with the BioPhase 8800 system, a multi-capillary electrophoresis system.

Two-dimensional separation AF4-UHPLC of macromolecules in 4 autochthonous grape varieties.

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The South-West region is unique in having a wide range of grape varieties (over 300), 120 of which being indigenous. The two main wine-growing regions in south-west France are the Gers (20,362 hectares, 2022) and the Tarn (6,274 hectares, 2022). Both of which produce dry white wines from typical south-western grape varieties: Colombard, Gros Manseng, Mauzac and Len de léI. Wines from each of these varieties have their own sensory identity. To differentiate them at macromolecular level, Asymmetrical Flow-Field Flow fractionation (AF4) was used. However, this method can only separate wine macromolecular populations by size.

The aim of this work was to identify the composition of each population in white wines by developing an 2D AF4-UHPLC system.

For this purpose, 69 wines were collected and first analyzed by Fourier Transform Infrared Spectroscopy (FTIR) to determine the conventional enological parameters (alcohol content, titratable acidity, pH…). After statistical processing by hierarchical ascending classification, 3 wines were selected for each variety. These wines were injected in AF4 coupled with UHPLC. To validate the system, two standards (thaumatin and mannoproteins) naturally present in the wines were injected. The results obtained in 2D were processed using LC image software in the form of mapping.

The results showed that each variety had its own 2D mapping. Colombard and Gros Manseng grape varieties showed different types of proteins, with varying degrees of affinity with the column. On the other hand, Len de l'eI and Mauzac varieties stood out for their total absence of proteins but their presence of mannoproteins.

As a perspective, fractions were collected to be analyzed by mass, NMR, etc.: This additional study to deeper understand macromolecules properties and their impact on the taste perceptions of these white wines.

Nanoplastic Quantification via Quartz Crystal Microbalance: An Approach for Hyphenation with Field-Flow Fractionation

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Nanoplastics (plastic particles smaller than 1 μm) are considered an abundant environmental contaminant and their impact on ecosystems is still not fully understood. This is partly because their analysis (identification and, especially, reliable quantification) is still challenging. While particle number-based quantification can be performed, for example, using nanoparticle tracking analysis (NTA) or dynamic light scattering (DLS), mass-based concentrations of nanoplastics can be derived from mass spectrometric techniques or a quartz crystal microbalance (QCM). A QCM consists of a piezoelectric crystal (quartz) which changes its resonance frequency with changes in mass deposited on its surface. Sensitivity down to the low nanogram range can be achieved depending on the specific setup. The advantage of this technique is that it solely relies on mass changes independent of any other material properties, which means that any particle can be detected with the same sensitivity (unlike with mass spectrometric techniques). This, however, requires that the specificity for nanoplastics is determined with another technique. While many studies use coated sensors to introduce chemical sensitivity for molecular analytes, this is not applicable to particles. Hyphenation with field-flow fractionation (FFF) can play an important role (also in combination with Raman microspectroscopy (RM)) to achieve the needed selectivity. FFF can be used for sample purification and size fractionation which could allow for correlation of the mass changes to different size classes. Furthermore, online coupled RM could provide the necessary size-resolved material identification within one measurement. In this work, the hyphenation of FFF and QCM was implemented using a 3D-printed microfluidic sprayer. The sprayer is used to nebulize part of the FFF detector flow with nitrogen and therefore to enhance the evaporation of the solvent on the sensor. This way, only the particles and the non- evaporating part of the surfactant is detected. A flow splitter is used to generate a very low flow rate (hundreds of μL/h) for the sprayer. Overall, the applicability for nanoplastic quantification will be evaluated using different particle sizes and materials at varying concentrations to show the independency of QCM towards other material properties besides mass.

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