Interface structure of 70% fish oil-in-water emulsions stabilized with combinations of sodium caseinate and phosphatidylcholine: use of small angle neutron and X-ray scattering techniques

Yesiltas, Betül; Torkkeli, Mika; Almasy, Laszlo; Dudás, Zoltán; Wacha, András Ferenc; García Moreno, Pedro Jesús; Sørensen, Ann-Dorit Moltke; Jacobsen, Charlotte; Knaapila, Matti

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Sydney | Australia
16 - 19 October 2018

Workshop hosted by ANSTO
Contents

Welcome 6
Overview 8
Program 10
Invited speakers 14
Map 16
Committees 18
Abstracts 20

DAY 1
Importance and problems of water in food and food related materials | Jan Swenson 20
From wetting to wear: visualization of contact zone in beverage tribology | Stefan Baier 21
Combining rheology and small-angle scattering of neutrons and X-rays for dynamic assessment of microfibrillated cellulose under shear | Evgenii Velichko 22
Neutron and X-Ray Reflectivity from chocolate sandwiches | Iva Manasi 23
Rheo-ND: Temperature and shear induced crystal transformation of a model triglyceride observed using neutron diffraction | Norman Booth 24
Determining seafood provenance and safety through nuclear techniques | Deboshish Mazumder 25
The interaction of native and modified clupeine with Gram-negative model membranes | Marcia English 26
Interaction and stability evaluation of chicken meat treated with gamma irradiation and turmeric powder | Muhammad Arshad 27

DAY 2
Lipid digestion – Key to the ability of milk-like emulsions to promote oral drug delivery | Andrew Clulow 34
Oil-in-water emulsion system stabilized by emulsion droplets coated with whey protein microgels | Lirong Cheng 35
Static and dynamic multiscale characterisation of micronized fat crystal network formation and disruption by USAXS and rthe-SAXS | Tatiana Nikolova 36
Heavy food molecules from the National Deuteration Facility for structure function applications | Tamim Darwish 37

DAY 3
The potential of scattering techniques to investigate the structure and molecular interactions of polysaccharides | Marta Martinez-Sanz 50
Brush-like polysaccharides with motif-specific interactions | Gleb Yokubov 51
Supramolecular characterisation of starch in rice by NMR, SAXS and XRD | Matthew Van Leeuwen 52
If we could design plant protein structures and tune properties in processed food | Ramune Kuktaite 53
Neutron scattering study of lipid sponge-phase nanoparticles as enzyme carriers for food processing | Tommy Nylander 54
A combined SANS and USANS study to investigate the structure of solid lipid nanoparticles | Rohan Shah 55
Structural investigation on nanostructured lipid carriers for fish oil by small angle scattering | Martin Schmiegel 56
Neutrons for industry - AONS ILO, ANSTO - Access, services and case studies | Anna Paradowska 57

Delegates 58
Welcome to Neutrons and Food 5 and Sydney

On behalf of the local organising committee, it is my pleasure to welcome you to the 5th International Neutrons and Food conference and the beautiful city of Sydney, Australia.

This is the latest in a successful series of conferences on the application, science and technology of neutron scattering techniques as applied to food materials, first held in Sydney in 2010. The conference has been held every two years since with attendees showcasing how neutron scattering techniques have been used, often combined with other complementary measurement methods including X-ray and light scattering. I am privileged to have the opportunity to chair this event and that this conference has returned to Australia.

I would like to take this opportunity to welcome all of our delegates, especially those in the early stages of their career, some of whom will be showcasing their research at this meeting. I would also like to sincerely thank all of our sponsors; the conference would not have been possible without your generous support.

Neutrons and Food 5 offers all delegates a stimulating scientific programme that covers no fewer than ten themes addressing both traditional topics as well as emerging applications. With neutron scattering remaining at its core, we will also showcase nuclear-based techniques more broadly and how they are increasingly being used.

I wish to extend a special welcome to our overseas delegates. I appreciate that many of you have travelled a long distance to share your expertise with us and I hope that you enjoy your stay here in Sydney. I would finally like to welcome those delegates attending the Neutrons and Food conference for the first time. I hope that you find the meeting valuable and that this will represent the beginning of a regular event in your calendar.

I wish you all an extremely successful conference, both personally and professionally.

Elliot Gilbert
Chair, Neutrons and Food 5
Overview

<table>
<thead>
<tr>
<th>Tuesday 16 October</th>
<th>Wednesday 17 October</th>
<th>Thursday 18 October</th>
<th>Friday 19 October</th>
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<tbody>
<tr>
<td>Optional tour: ANSTO</td>
<td>Optional tour: Symbio Wildlife Park</td>
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<tr>
<td><strong>DAY 1</strong></td>
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<td>Registration</td>
<td>Food emulsions</td>
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<td>Neutrons and Food Welcome</td>
<td>Lipids and fats</td>
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<td>11.00 am</td>
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<td>Glassy states</td>
<td>Dairy</td>
<td>IUCr Travel Award Presentation</td>
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<td>Processing, rheology and tribology</td>
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<td>Lunch</td>
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# Program

## Tuesday 16 October

<table>
<thead>
<tr>
<th>Time</th>
<th>Session</th>
<th>Speaker(s)</th>
<th>Affiliation</th>
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<td>9.00 am</td>
<td>Registration</td>
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<td>9.30 am</td>
<td>Morning tea</td>
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<td>45 mins</td>
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<tr>
<td>10.15 am</td>
<td>Neutrons and Food Welcome</td>
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<td>25 mins</td>
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</tbody>
</table>

### MORNING SESSION | Glassy states

10.40 am | Importance and problems of water in food and food related materials | Jan Swenson | Chalmers University of Technology | 40 mins |

### MID MORNING SESSION | Processing, rheology and tribology

11.20 am | From wetting to wear: visualization of contact zone in beverage tribology | Stefan Raier | PepsiCo and University of Queensland | 20 mins |

11.40 am | Combining rheology and small-angle scattering of neutrons and X-rays for dynamic assessment of microfibrillated cellulose under shear | Evgenii Velichko | TU Delft | 20 mins |

12.00 am | Neutron and X-Ray reflectivity from chocolate sandwiches | Iva Manasi | University of Edinburgh | 20 mins |

12.20 am | Combining rheology and small-angle scattering of neutrons and X-rays for dynamic assessment of microfibrillated cellulose under shear | Evgenii Velichko | TU Delft | 20 mins |

### Midnoon - Lunch

Lunch - Sponsored by Riddet Institute | 1 h 20 mins |

### MID AFTERNOON SESSION | Food packaging, preservation, safety and quality

2.00 pm | Determining seafood provenance and safety through nuclear techniques | Debashish Hazamder | ANSTO | 20 mins |

2.20 pm | The interaction of fluorinated compounds with a phospholipid bilayer | Shirin Nouhi | Uppsala University | 20 mins |

2.40 pm | Interaction of native and modified clupeine with Gram-negative model membranes | Marcia English | Saint Francis Xavier University | 20 mins |

3.00 pm | Quality and stability evaluation of chicken meat treated with gamma irradiation and turmeric powder | Muhammad Arshad | Government College University Faisalabad, Pakistan | 20 mins |

3.20 pm | Afternoon tea - Sponsored by ANBUG | | 30 mins |

### AFTERNOON SESSION | Innovative methods for food (nuclear, synchrotron and light)

3.50 pm | Neutrons and food - an introduction | Elliot Gilbert | ANSTO | 20 mins |

4.10 pm | Applications of Neutron Activation Analysis in food studies | Attila Stopic | ANSTO | 20 mins |

4.30 pm | Opportunities for QENS and neutron spectroscopy at ACNS | Nicolas de Souza | ANSTO | 20 mins |

4.50 pm | High-resolution macro ATR-FTIR chemical imaging capability at Australian Synchrotron IR Beamline and its applications in food science | Nils Ingvarsson | ANSTO | 20 mins |

5.10 pm | X-ray fluorescence microscopy capabilities at the Australian Synchrotron and applications in food science | David Paterson | ANSTO | 20 mins |

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**7.30 pm** | Welcome Reception | Casa Ristorante | 2 h |

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## DAY 1

**Wednesday 17 October**

<table>
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<tr>
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**Thursday 18 October**

<table>
<thead>
<tr>
<th>MORNING SESSION</th>
<th>Food emulsions</th>
<th>CHAIR</th>
<th>Tommy Nylander</th>
<th>Li Xing Chang</th>
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<tbody>
<tr>
<td>9.00 am</td>
<td>Lipid digestion – Key to the ability of milk-like emulsions to promote oral drug delivery</td>
<td>20 mins</td>
<td>Andrew Glazier</td>
<td>Monash University</td>
</tr>
<tr>
<td>9.20 am</td>
<td>Oil-in-water emulsion system stabilized by emulsion droplets coated with whey protein microgels</td>
<td>20 mins</td>
<td>Li Xing Chang</td>
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</tr>
<tr>
<td>9.40 am</td>
<td>Interface structure of 70% fish oil-in-water emulsions stabilized with combinations of sodium caseinate and phosphatidylcholine</td>
<td>20 mins</td>
<td>Beatrix Yeates</td>
<td>National Food Institute, Technical University of Denmark</td>
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<tr>
<td>10.15 am</td>
<td>Open Forum - Facilitator: Jamie Schulz</td>
<td>40 mins</td>
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<tr>
<td>10.45 am</td>
<td>Morning tea - Sponsored by ACNS</td>
<td>40 mins</td>
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<table>
<thead>
<tr>
<th>MID MORNING SESSION</th>
<th>Lipids and fats</th>
<th>CHAIR</th>
<th>Hitoshi Sato</th>
<th>Beatrix Yeates</th>
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</thead>
<tbody>
<tr>
<td>11.20 am</td>
<td>The memories of liquid triacylglycerols</td>
<td>20 mins</td>
<td>Gianfranco Mazzanti</td>
<td>Dalhousie University</td>
</tr>
<tr>
<td>11.40 am</td>
<td>Structure formation in oleic acid - sodium oleate based oleogels</td>
<td>20 mins</td>
<td>Steven Cornet</td>
<td>Wageningen UR</td>
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<tr>
<td>12.00 am</td>
<td>Static and dynamic multiscale characterisation of micromized fat crystal network formation and disruption by USAXS and rheo-SAXS</td>
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<td>Tatiana Nikolova</td>
<td>Wageningen UR</td>
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<th>Innovative methods for food (nuclear, synchrotron and light)</th>
<th>CHAIR</th>
<th>Hitoshi Sato</th>
<th>Beatrix Yeates</th>
</tr>
</thead>
<tbody>
<tr>
<td>12.20 pm</td>
<td>Heavy food molecules from the National Deuteration Facility for structure function applications</td>
<td>20 mins</td>
<td>Tamim Darwish</td>
<td>ANSTO</td>
</tr>
<tr>
<td>12.40 pm</td>
<td>Lunch - Sponsored by Office of NSW Chief Scientist</td>
<td>1 h 20 mins</td>
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<tr>
<th>MID AFTERNOON SESSION</th>
<th>Dairy</th>
<th>CHAIR</th>
<th>Hitoshi Sato</th>
<th>Shinya Nakahara</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.00 pm</td>
<td>Structural characterization of milk coagulation from 0.1 to 20μm using Ultra-Small Angle Neutron Scattering</td>
<td>40 mins</td>
<td>Alejandro Marangoz</td>
<td>University of Guelph</td>
</tr>
<tr>
<td>2.40 pm</td>
<td>Ultra-Small Angle Neutron Scattering investigation of milk coagulation: Data analysis and contrast matching methods</td>
<td>20 mins</td>
<td>Nukhalu Callaghan-Patrachar</td>
<td>University of Guelph</td>
</tr>
<tr>
<td>3.00 pm</td>
<td>Dynamics of calcium caseinate in H2O or D2O studied by Quasi-Elastic Neutron Scattering (QENS)</td>
<td>20 mins</td>
<td>Bet Tin</td>
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<tr>
<td>3.20 pm</td>
<td>Afternoon tea - Sponsored by Anton-Paar</td>
<td>30 mins</td>
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<tr>
<td>3.50 pm</td>
<td>Ultra-small angle neutron scattering studies on milk and cheese curd formation</td>
<td>20 mins</td>
<td>Carl Adams</td>
<td>St. Francis Xavier University</td>
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<tr>
<td>4.10 pm</td>
<td>Structure of Artificial Casein Micelles Composed of Deuterated β-Casein and Native κ-Casein: A Contrast Variation SANS and SAXS Study</td>
<td>20 mins</td>
<td>Jared Raynes</td>
<td>CSIRO</td>
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**DAY 3**
**Friday 19 October**

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<tr>
<th>MORNING SESSION</th>
<th>Plant materials</th>
<th>CHAIR</th>
<th>Marianne Gaborieau</th>
<th>Ugur Yesiltas</th>
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<tr>
<td>9.00 am</td>
<td>The potential of scattering techniques to investigate the structure and molecular interactions of Polycaccharides</td>
<td>40 mins</td>
<td>Marta Martinez-Sanz</td>
<td>IATA, CSIC</td>
</tr>
<tr>
<td>9.40 am</td>
<td>Brush-like polysaccharides with motif-specific interactions</td>
<td>20 mins</td>
<td>Gleb Volikov</td>
<td>University of Queensland</td>
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<tr>
<td>10.00 am</td>
<td>Supramolecular characterisation of starch in rice by NMR, SAXS and XRD</td>
<td>20 mins</td>
<td>Matthew Van Leeuwen</td>
<td>Western Sydney University</td>
</tr>
<tr>
<td>10.20 am</td>
<td>If we could design plant protein structures and tune properties in processed food</td>
<td>20 mins</td>
<td>Ramee Ukkanta</td>
<td>Swedish University of Agricultural Sciences</td>
</tr>
<tr>
<td>10.40 am</td>
<td>Morning tea</td>
<td>40 mins</td>
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<th>MID MORNING SESSION</th>
<th>Encapsulation and controlled release</th>
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<th>Andrew Glazier</th>
<th>Nukhalu Callaghan-Patrachar</th>
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<tr>
<td>11.20 am</td>
<td>Neutron scattering study of lipid sponge-phase nanoparticles as enzyme carriers for food processing</td>
<td>20 mins</td>
<td>Tommy Nylander</td>
<td>Lund University</td>
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<tr>
<td>11.40 am</td>
<td>A combined SANS and USANS study to investigate the structure of solid lipid nanoparticles</td>
<td>20 mins</td>
<td>Rohan Shah</td>
<td>Windermere University of Technology</td>
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<tr>
<td>12.00 am</td>
<td>Structural investigation on nanostructured lipid carriers for fish oil by small angle scattering</td>
<td>20 mins</td>
<td>Martin Schmiedl</td>
<td>Rhenenbahn University</td>
</tr>
<tr>
<td>12.20 pm</td>
<td>IUC Travel Award Presentation</td>
<td>40 mins</td>
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<tr>
<td>1.00 pm</td>
<td>Lunch - Sponsored by ANSTO</td>
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<table>
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<th>CHAIR</th>
<th>Jitendra Mata</th>
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<tr>
<td>2.00 pm</td>
<td>Neutrons for Industry - ACNS ILO, ANSTO - Access, services and case studies</td>
<td>20 mins</td>
<td>Anna Paradowska</td>
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<tr>
<td>2.20 pm</td>
<td>Industry Q&amp;A - Facilitator: Jitendra Mata</td>
<td>40 mins</td>
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<tr>
<td>3.00 pm</td>
<td>Neutrons and Food 6 Presentation</td>
<td>20 mins</td>
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**DINNER CRUISE**
Harbourside Cruises
Invited speakers

**Prof Alejandro Marangoni**
University of Guelph

Prof Alejandro G. Marangoni is a professor and Tier I Canada Research Chair Food, Health and Aging at the University of Guelph, Canada. Alejandro’s work concentrates on the physical properties of lipidic materials in foods, cosmetics and biolubricants. He has published over 350 refereed research articles, 60 book chapters, 13 books, and over 40 patents. He is the recipient of many awards including the 2013 AOCS Stephen Chang award, the 2014 IFT Chang Award in Lipid Science, the 2014 Supelco/Nicholas Pelick Award, the 2015 ISF Kaufmann Medal and the 2017 Alton E. Bailey Award of the AOCS. Marangoni was honored as one of the 10 most influential Hispanic Canadians in 2012 and a Fellow of the American Oil Chemists’ Society in 2015.

Prof Jan Swenson
Chalmers University of Technology

Jan was born in 1966 and earned his PhD in Physics at Chalmers University of Technology, Sweden, in 1996. He was thereafter a post-doctoral fellow at University College London, UK, the following two years, before he returned to Chalmers for an assistant professorship.

Jan was also employed by the Royal Swedish Academy of Sciences before he got a full professorship at NTNU, Norway, in 2005. Since 2006 he has held his current position as professor in physics at Chalmers. His current main research interest concerns the role of water in biological materials and how biological materials can be stabilized and cryopreserved by a cryoprotective substance, such as trehalose.

Jan is also working on the development of new simulation methods to model neutron scattering data. He has published about 150 reviewed scientific papers and has a H-index of 42.

**Dr Marta Martinez-Sanz**
Spanish National Research Council (CSIC)

Marta received a BSc (Hons) in Chemical Engineering and a Ph.D. in Food Science at the Polytechnic University of Valencia, Spain. She is currently a Juan de la Cierva fellow at the Food Quality and Preservation Department at the Institute of Agrochemistry and Food Technology (IATA-CSIC) (Spain).

Marta’s research focuses on the extraction and characterisation of poly saccharides from renewable resources, with special focus on aquatic biomass, for the production of food additives and food packaging materials. Prior to this position, she worked for three years as a joint post-doctoral research fellow in the Australian Nuclear Science and Technology Organisation (ANSTO) and the University of Queensland (UQ). Marta’s research focused on the investigation of the multi-scale structure of plant cell walls and model systems utilizing small angle scattering and diffraction techniques in combination with complementary microscopy, spectroscopy and rheology methods.

Marta carried out her PhD at the Institute of Agrochemistry and Food Technology (IATA-CSIC), specializing in the synthesis and characterisation of bio-based nanofillers extracted from renewable resources, such as plant-derived and bacterial cellulose nanocrystals and nanokertins, as well as on the development of novel routes to incorporate them into polymeric matrices. Overall, her research is focused on the multi-scale structural investigation of bio-based materials and food-based systems, making use of a wide range of techniques and advanced characterization tools.
World leading research for tomorrow’s innovations in food

The Riddet Institute is a New Zealand Centre of Research Excellence, with a world-leading reputation for fundamental and strategic scientific research in nutrition, food science and technology. We combine experienced scientists with emerging researchers, to create unique expertise from a range of disciplines and organisations. Our 190 researchers across New Zealand contribute to scientific programmes that are advancing the frontiers in nutrition and food science.

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From wetting to wear: visualization of contact zone in beverage tribology

Matching the mouthfeel of sugar is still one of the largest unsolved challenges of product developers, as sugar not only provides sweetness, but also mouthfeel. Mouthfeel is one of the most critical product attributes driving consumer choice and acceptability of reduced sugar beverages. In order to link mouthfeel attributes to analytical measures, one must first of all appreciate that drinking is a highly dynamic process that involves mixing of the ingested beverage with saliva and the resulting bolus being sheared over several length scales between the tongue and the upper palate. In order to better interpret saliva driven changes to beverage bolus, it is important to recognize the conversion of the beverage from a simple Newtonian fluid to a multiphase complex fluid. Currently, our ability to properly interpret tribology data from multiphase complex fluids is rather limited, as we lack additional information on structure (atomic, molecular, macromolecular) in the contact zone. Therefore developing techniques that will allow us to measure structural changes governed by saliva beverage interactions will lead to better insights to enable the rational design of reduced sugar beverages.

The authors are employed by PepsiCo, Inc. The views expressed in this abstract are those of the authors and do not necessarily reflect the position or policy of PepsiCo, Inc.

Authors
Stefan Baier 1,2
Michael Boehm 2
1 University of Queensland
2 PepsiCo

Corresponding author
stefan.baier@pepsico.com

Abstracts

Importance and problems of water in food and food related materials

Most food contains relatively large amounts of water and this water is generally of central importance for the taste and other properties of the food. However, in the case of long-time storage this water can be problematic and cause a lot of detrimental aging effects. One way to overcome such aging effects is to reduce the temperature and thereby slow down the aging, but this gives often rise to ice formation, which generates other problems of damaged cellular structures in the food materials. To overcome this problem it is generally required to dry the food material before it is long-time stored at a low temperature, so called freeze-drying. Nevertheless, water in food materials, as well as in living biological materials, is both essential and sometimes problematic. In this presentation these important and detrimental roles of water in carbohydrate rich food will be discussed. For example, we have elucidated the nature of ice crystals in frozen dough and bred [1], and how these ice crystals grow with increasing storage-time [2,3]. We also discuss attempts to reduce the detrimental ice formation. Furthermore, we have investigated how the dynamics of the water in carbohydrate rich food is affected by the structure and/or dynamics of the food material. It was found that the water dynamics becomes slower in food materials with slow dynamics, which implies that the dynamics of the water and the pure food material are interrelated [4]. It was also evident that the water dynamics speeds up dramatically with increasing water content [4]. Finally, the anomalous dynamical properties of deeply supercooled water will be discussed, and how this affects the freeze-storage of biological materials.

Combining rheology and small-angle scattering of neutrons and X-rays for dynamic assessment of microfibrillated cellulose under shear

Cellulose fibre is a natural food component present in fruits and vegetables. Presence of the cellulose fibre in the diet aids digestion and helps in preventing a variety of diseases. Cellulose fibres also provide texture to food products. The texturing capacity of cellulose fibres can be enhanced by defibrillation under high shear. Dispersions of microfibrillated cellulose form a 3D soft space-filling network, which can, for example, mimic the texture of fat-based products. Critical for the consumer perception of microfibrillated cellulose networks are spreadability and mouthfeel. The microstructural features that underly these rheological parameters are poorly understood. Small-angle scattering of neutrons (SANS) and X-rays (SAXS) provides structural information in a wide range of length scales. Combining of rheological data with SANS/SAXS measurements of the samples under shear conditions sheds light on the mesoscale structural dynamics that underlie the peculiar flow of microfibrillated cellulose under industrial processing conditions and during consumer use and consumption.

Recent studies [1] have shown nonlocal flow behaviour of microfibrillar cellulose suspensions. Based on Rheo-MRI data two different hypotheses to explain such nonlocality were suggested: flocculation of cellulose microfibrils and formation of a liquid-crystal like phase in these suspensions. To validate these hypotheses we constructed a special sample cell, which allows for SANS/SAXS on the materials under shear with spatial resolution (100 μm) across the velocity gradient direction of the cell. No alignment of microfibrills was observed at shear rates from 1 to 300 s⁻¹. The scattering invarient calculation indicates a homogeneous density distribution of cellulose microfibrils across the gap at all applied shear-rates. This implies that no shear-induced aggregation and consequent migration of cellulose over the gap occurs. The shear-SAXS experiments are in line with the flocculation hypothesis with the flock sizes much larger than 200 nm (maximal size in the performed experiment).


Neutron and X-Ray reflectivity from chocolate sandwiches

Chocolate is a dense suspension of solids, mainly sucrose, in a continuous triglyceride fat phase of cocoa butter, containing Lecithin, which is mostly phospholipid, and in some cases the polymeric surfactant PolyGlycerol PolyRicinoleate (PGPR). These surfactants reduce the yield stress and viscosity of molten chocolate, which is important in chocolate manufacture and influences the mouth feel of the chocolate. How these surfactants cause these modifications to the rheology of molten chocolate remains an open question.

We have developed a methodology that allows the preparation of well-defined crystalline planar sucrose films as substrates to study the structures formed by these surfactants at sucrose/triglyceride interfaces. X-ray and neutron reflectivity has been used in combination with QCM-D to characterize the adsorption of these surfactants individually and in combination at the sucrose/triglyceride interface to provide a structural basis for the observed rheology.


Keyword: Rheology, interfacial structure, reflectivity, triglyceride
Rheo-ND: Temperature and shear induced crystal transformation of a model triglyceride observed using neutron diffraction

Rheo-SANS (rheology and small angle neutron diffraction) is now a well-established technique to probe the shape and size of particles under different shear and temperature regimes. Recent work on WOMBAT 2, the high intensity neutron diffraction instrument at the Australian Centre for Neutron Scattering, has successfully combined rheology and neutron diffraction for the first time. This was used to follow the crystalline phase transformations in a model (deuterated) triglyceride.

The initial impetus for this work was part of a forensic investigation linking the crystallisation of triglycerides under high shear rates that are encountered in motor vehicle accidents. However, now the technique has been demonstrated it may be of interest to investigate fat crystallisation due to shear in food manufacture. Possible applications might include investigation of detrimental crystallisation during conching or tempering of chocolate or extrusion of foods high in fats (string cheese?).

The high neutron flux and detector efficiency available at WOMBAT is instrumental in allowing diffraction patterns to be collected from the small sample volumes available in the Couette cell geometry. The Couette cell used has a sample gap of 1mm and was mounted tangentially to the neutron beam. The shear rate applied to the sample can be varied over a range of 10-2500 s⁻¹ and the temperature can be controlled over a range of -10 and 80 °C. We invite other researchers who are interested in this capacity to contact us about possible experiments.


Determining seafood provenance and safety through nuclear techniques

Demand for farmed or wild-caught seafood is growing globally. Regulatory bodies, market chain actors and consumers have an interest in seafood provenance and food safety assessment tools to protect human health, guarantee sustainability, meet certification requirements, reduce or prevent food fraud, and to minimise biosecurity risks associated with global movement of raw seafood products. The limitations of current food safety and quality assessment methods are being overcome using complementary or standalone nuclear techniques. Food quality is a complex term and has often been associated with food safety by consumers; traceability, a key requirement of the global seafood trade, is linked to both food safety and quality. The study investigated tracability and food quality using nuclear techniques. The traceability component focused on determining the geographical origin and production method of Asian seafood (Lates calcarifer) and black tiger prawns (Penaeus monodon) from a range of Asia-Pacific locations using Stable Isotope Analysis (SIA) and X-ray fluorescence (XRF) through Itrax. The food quality component focused on using Neutron Activation Analysis (NAA) to detect the concentration of heavy metals and other elements (39 in total) in the two species.

The data were analysed using three different statistical methods: univariate and multivariate analyses, Linear Discriminant Analysis (LDA), and Random Forest. Both LDA and random forest had consistent results: XRF effectively distinguished the production method and geographic locations of P. monodon (up to 100% accuracy), and SIA was more effective for L. calcarifer (up to 94% accuracy). In most cases for both L. calcarifer and P. monodon, NAA revealed that the concentrations of heavy metals and micronutrients were below the threshold levels of concern for human consumption. There were no significant differences between farmed and wild-caught samples or between geographic locations. However, in some cases, elements such as arsenic and selenium were significantly higher than the threshold, but their chemical forms are yet to be determined.

SIA and XRF are effective complementary methods for determining the provenance of seafood. NAA should be combined with other methods such as protein analysis and fatty acid profiling to effectively determine the quality of seafood.
The interaction of fluorinated compounds with a phospholipid bilayer

Some industrial surfactants that are commonly used in preparation of non-stick coatings for kitchen dishes and in food packaging products are fluorinated compounds as they have good water and stain repellent properties. These materials however now attract increasing global concern due to their persistence, bioaccumulation and possible adverse effects on the environment and living organisms.

Concerns regarding these molecules being carcinogenic and possibly harmful for children immunes system have been raised. The use of some of these compounds, in particular long-chain compounds, has been banned in many countries and some other compounds are under discussion as regards continued use. In the past few years, new alternative molecules with shorter chain length or with partly fluorinated chains have been introduced as replacements that are thought to be safer products.

There is however little known about the interaction of these compounds with biological systems or well-founded reasons for specific choices. In this project, we have investigated the interaction of fully and partly fluorinated compounds with model systems relevant to biological interfaces using neutron reflectometry. Neutrons provide a unique and powerful tool to investigate the amount and location of fluorocarbons when mixed with hydrophobic molecules.

The study covers a range of compounds, from the ones that are already banned to new replacement alternatives. This allows a comparison of the effect of hydrophobic chain length and of different hydrophilic functional groups on their interactions with phospholipid bilayers.

Fully fluorinated molecules have been shown to penetrate into bilayers and displace the lipids whereas partly fluorinated compounds show negligible interactions with the lipid. Off-specular scattering data from bilayers which have been contacted with fully fluorinated compounds indicate that there are rough and patchy structures after exposure.

Extensive rinsing with water removes some of the fluorocarbon surfactant but a less dense lipid bilayer is left behind.

The interaction of these compounds has been found to vary with the head group as: sulfonamide > sulfonate > carboxylic acids and to increase with the chain length [1] which strongly correlates with their solubility in water.


Interaction of native and modified clupeine with Gram-negative model membranes

Clupeine, a cationic antimicrobial peptide found in the sperm cells of fish, is of interest as a food additive because of its antimicrobial activity against several foodborne pathogens. However, it has previously been shown that non-specific binding of clupeine to anionic molecules reduces its antimicrobial activity. It has also been shown that the overall positive charge of the native peptide can be reduced by blocking 10% of its arginine residues with 12-cyclohexanedione (CHD) to form CHD-treated clupeine. CHD-treated clupeine retains antimicrobial activity but it is not known if the modes of interaction against Gram-negative bacteria remain the same as the native peptide. The focus of the present study was to investigate the effect of charge reduction on peptide membrane interactions by comparing the effect of native and CHD-treated clupeine on Escherichia coli (E. coli) model biomembranes.

It was hypothesized that the reduction in charge would result in different interactions with model monolayers composed of E. coli PE, phosphatidylethanolamine, 79 mole% PC, phosphatidylglycerol, 17 mole% and CL, cardiolipin, 4 mole% and modified bilayer membranes composed of DPPC, PEGCL (79/17 mole%).

Peptide interaction with the model biomembranes were studied using Neutron Reflectometry (NR) and X-ray reflectometry (XRR) and symmetric bilayers were deposited on silicon blocks applying the Langmuir- Blodgett and Langmuir Schaefer techniques. Some lipid mixing was observed in the inner tail region (~69 ± 0.24% DPPC, 12-dipalmitoyl (d62)-snglycero-3-phosphocholine) and ~24 ± 0.02% PE:PG:CL) and in the outer tail region (~20 ± 0.02% DPPC and ~56 ± 0.01% PE:PG:CL).

Native and CHD-treated clupeine were not able to cross the model PE:PG:CL:DPPC bilayer biomembrane, however, CHD-treated clupeine showed increased interactions with the lipid head group. Inspite of the different interactions observed in the model systems, a more comprehensive understanding of the safety and toxicology of both peptides is required before they can be used for food applications.
Neutrons and Food – an Introduction

The application of neutron scattering methods to understand the structure and dynamics in soft-condensed matter has a long history but its utilisation for food-based materials still remains the domain of a small, albeit increasing, community. This is despite the fact that neutrons offer significant advantages over other avenues of characterisation where an understanding at the nanoscale is essential to explain the functional properties and behaviour in food. Neutrons are isotopically sensitive and are also highly penetrating enabling transmission through complex sample environments; the latter provides the potential to study industrially-relevant processes in real time. The presence of higher flux facilities, with access to extended spatial and energy ranges, greater computational power and enhanced modelling methods, offer further opportunities. This presentation will provide a brief introduction to neutron scattering and how such methods can yield useful information on food materials.

Quality and stability evaluation of chicken meat treated with gamma irradiation and turmeric powder

The safety of chicken meat increased by treatment of gamma radiation. The free radicals produced due to radiation in food products. To overcome this issue, some source of antioxidants used along with radiation. Turmeric powder has the ability to work as antioxidant as well as antimicrobial. The study was carried out to evaluate the impact of gamma irradiation and turmeric powder (TP) on microbial quality, physicochemical quality, stability and antioxidant status of chicken meat. There were 2 doses (1 kGy and 2 kGy) of gamma irradiation was used alone and with combination of 3% turmeric powder along with control (0kGy). Aerobic and vacuum packaging were used for the storage of chicken meat with interval of 0, 7 and 14 days at 4°C.

The microbiological results showed that the level of contamination was in the decreasing order as the dose of gamma irradiation increases both for total bacteria and coliforms, whereas no contamination was found in group treated with 2 kGy+TP both for aerobic and vacuum packaging. The peroxide value (POV), thiobarbituric acid reactive substances (TBARS) and total volatile basic nitrogen (TVBN) differed significantly on chicken meat with different groups. Higher POV and TBARS were found in chicken meat treated with 2 kGy under aerobic packaging after 14 days of storage and TVBN was higher in control at day 14 of storage under aerobic packaging whereas minimum POV, TBARS and TVBN were found in 0kGy+TP at day 0 under vacuum packaging.

The results showed that different sensory attributes were evaluated by the panel of judges and higher score for all the sensory attributes like appearance, taste, texture, flavor and overall acceptability was found in 2 kGy. It is concluded that the chicken meat treated with 2 kGy+TP was considered better for microbial and physicochemical quality, antioxidant activity as well as sensorial properties of chicken meat.

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Applications of Neutron Activation Analysis in food studies

Neutron Activation Analysis (NAA) is a sensitive technique for quantifying elemental concentrations in solid samples. It is a mature and well-understood method that has been in use for several decades across a range of disciplines. Today it is one of many techniques available to analysts for characterising the elemental makeup of solid samples, albeit with somewhat limited availability as a powerful neutron source, such as a nuclear research reactor, is a requirement. Partly but not entirely as a result of this, it is often overlooked in favour of more readily accessible methods, yet still holds some key advantages over other methods commonly used. This talk aims to cover the basic concepts of NAA and outline the advantages and disadvantages in the context of elemental analysis of food, while outlining the experience of the NAA capability at the Australian Nuclear Science & Technology Organisation (ANSTO).

AUTHOR
Attila Stopic 1
1 ANSTO

CORRESPONDING AUTHOR
attila.stopic@ansto.gov.au

Opportunities for QENS and neutron spectroscopy at ACNS

The Australian Centre for Neutron Scattering presently operates two neutron inelastic spectrometers well suited to quasi-elastic neutron scattering (QENS) and low-energy neutron spectroscopy studies. In combination, the EMU and PELICAN cold-neutron spectrometers cover an energy transfer range spanning 0.001 to 14 meV. As far as QENS, the accessible time domain is thus from pico- to nano- seconds, with a momentum transfers ranging from 0.1 to beyond 2 Å⁻¹.

The main features of the two instruments will be presented, including their available sample environments. EMU is a backscattering spectrometer, and PELICAN is a polarised time-of-flight spectrometer. Examples highlighting the capabilities of each spectrometer and their complementarity will be presented, with a focus on application areas such as soft-matter and bio-material relaxations, and aqueous/hydration dynamics.

AUTHOR
Nicolas de Souza 1
1 Australian Centre for Neutron Scattering, ANSTO

CORRESPONDING AUTHOR
nrlsouza@gmail.com
X-ray fluorescence microscopy capabilities at the Australian Synchrotron and applications in food science

X-ray fluorescence microscopy (XFM) can be used for elemental and chemical microanalysis across length scales ranging from millimeter to nanometer. XFM is ideally suited to quantitatively map trace elements within whole and sectioned plants, seeds, animals and soil samples. The high elemental sensitivity of X-ray fluorescence microprobes coupled with deep penetration of hard X-rays enables measurement of an incredibly diverse range of samples in situ and under environmental conditions with a minimum of preparation.

The ability to rapidly acquire 2D images enables higher-dimensional studies such as fluorescence tomography, X-ray absorption near edge structure (XANES) imaging, and XANES tomography in realistic times. Full spectral XANES imaging takes advantage of fast XFM and results in X-ray absorption near edge structure spectra from X-ray fluorescence at each pixel in the image. This enables spatially resolved chemical speciation and the efficiency and speed ensures the lowest possible dose alongside high throughput.

Examples of XFM elemental mapping addressing bio-fortification of cereal grains and chemical speciation studies to understand manganese and arsenic toxicity in plants and selenium bio-availability in aquatic environments will be presented.

AUTHOR

David Paterson
Australian Centre for Neutron Scattering, ANSTO

corresponding author

davidp@ansto.gov.au

High-resolution macro ATR–FTIR chemical imaging capability at Australian Synchrotron IR Beamline and its applications in food science

This work presents advances in high-resolution chemical imaging capability at Australian Synchrotron Infrared (IR) beamline, achieved through the use of an in-house developed synchrotron macro ATR-FTIR microspectroscopic device [1]. The device was developed by modifying the cantilever arm of a standard macro-ATR unit to accept germanium (Ge) ATR elements with different contact facet sizes (i.e. 1 mm, 250 μm and 100 μm in diameter). Coupling synchrotron-IR beam to the Ge ATR element (n=4) used in this device, has the effect of not only reducing the beam focus size (improving the lateral resolution) by a factor of 4, but also reducing the mapping step size by 4 times relative to the stage step motion. As a result, the macro ATR-FTIR measurement at Australian Synchrotron IR Beamline can be performed at minimum beam size of 19 μm using a 20x objective, and at minimum mapping step size of 250 nm, allowing high-resolution chemical imaging analysis.

While the large Ge facet size works well with soft materials that do not require high pressure to achieve a good contact, the small tips can provide higher pressure and allow measurements inside smaller regions with limited access suitable for hard and rough surfaces [1-2]. This macro ATR-FTIR device can also be coupled to a temperature control unit, allowing temperature-dependent study, as well as measurements that require a fixed temperature such as analysis of dairy products at 4°C similar to the usual storage condition in a household fridge.

The development of the macro ATR-FTIR device has so far led to successful analysis of samples from a diverse range of research disciplines. Key applications in food science to be presented include a range of dairy products (e.g. cheese and yoghurt), plants and vegetables.

AUTHORS

Jitraropn Vongsvivut
Anita Pax
Pyo Sekine
Enzo Lombi
Yakinda Prasad Timilsena
Benu Adhikari
Solly Gras
Mark Tobin

1 Australian Synchrotron, ANSTO
2 The University of Melbourne
3 University of South Australia
4 RMIT University

1 Australian Centre for Neutron Scattering, ANSTO
2 The University of Melbourne
3 University of South Australia
4 RMIT University

CORRESPONDING AUTHOR

pimm@synchrotron.org.au


Lipid digestion – Key to the ability of milk-like emulsions to promote oral drug delivery

Milk is a staple of the human diet and is an essential nutrient source for all mammalian infants. The fat content of milk comprises 98% triglycerides emulsified with milk fat globular membrane material and native proteins. Digestion of these triglycerides in the intestines yields monoglycerides and fatty acids that are absorbed through the intestinal wall. Lipophilic molecules such as drugs and nutrients can be dissolved in the milk fat globules but fat digestion can result in further dissolution or precipitation of incorporated drugs and nutrients. Digestion of milk fats also leads to the formation of intricate liquid crystalline structures that evolve throughout digestion,[1,2] which may also affect the release of incorporated drugs and nutrients. The process of lipid digestion is therefore key to the fate of lipophilic molecules dissolved in the fat globules of the milk emulsion and directly controls milk’s ability to act as an excipient for lipophilic drugs and nutrients.

In this presentation, small angle X-ray and neutron scattering techniques (SAXS and SANS, respectively) will be used to probe the structural transformations occurring during the digestion of milk fats and the subsequent effects on incorporated drugs. Synchrotron SAXS reveals that the liquid crystalline structural transformations occurring during the digestion of milk fats are robust with respect to heat treatment, freezing and powdering/reconstitution of milk.[3] It will also be established that milk-substrates such as infant formula and vegetable juices do not necessarily replicate milk-like structures during digestion. The distribution of digestion products within fat globules at different extents of digestion will be examined with SANS combined with selective deuteration of digestion products within fat globules at different extents of digestion.

The results showed that the WPM particles produced in the absence of phosphate buffer (WPM-NPB) were smooth spherical particles, giving a surface fractal dimension of 2.3 and a hydrodynamic diameter of 270 nm. However, WPM particles made in the presence of phosphate buffer (WPM-NPB) were smooth spherical particles, giving a surface fractal dimension of 2.0 and a hydrodynamic diameter of 250 nm. The surface fractal dimension of the WPM particles produced in the absence of phosphate buffer (WPM-NPB) was 2.3, and the hydrodynamic diameter was 270 nm. However, WPM particles made in the presence of phosphate buffer (WPM-NPB) were smooth spherical particles, giving a surface fractal dimension of 2.0 and a hydrodynamic diameter of 250 nm.

Oil-in–water emulsion system stabilized by emulsion droplets coated with whey protein microgels

Structurally designed emulsions are a developing group that is likely to find increasing utilization within the food industry because of their potential advantages over conventional emulsions. A novel droplet-stabilized (DS) emulsion system emulsified with casein micelles has been previously reported[1] However, the mechanism of DS emulsion formation, physicochemical properties, and their stability are not yet been fully explored. In the present study, heat-induced whey protein microgel (WPM) particles were used as an alternative emulsifying agent. The structure of WPM particles on the formation and physicochemical properties of the primary (PE) and the DS emulsions was investigated. WPM particles were prepared by heating 4 wt% whey protein isolate solution in the presence of 10 mM phosphate buffer at pH 5.9, 85°C for 45 min, followed by washing, centrifugation, and micro-fluidization. The PE coated with WPM were homogenized using 3 passes at the pressure of 250 bar. DS emulsions were prepared by mixing (at 3000 rev/min for 2 min) 10 wt% oil with 10, 30 or 60 wt% PE. The structure of WPM particles and emulsions were analyzed by dynamic light scattering confocal light scattering microscopy (CLSM), transmission electronic microscopy (TEM), and the combination of small and ultra-small angle neutron scattering (SANS and USANS).

The results showed that the WPM particles produced in the absence of phosphate buffer (WPM-NPB) were smooth spherical particles, giving a surface fractal dimension of 2.3 and a hydrodynamic diameter of 270 nm. However, WPM particles made in the presence of phosphate buffer (WPM-PB) were rough spherical particles with a surface fractal dimension of 2.3 and a hydrodynamic diameter of 290 nm. Particle fragments present in the WPM-PB dispersion, resulted in its competitive adsorption onto the surface of the DS emulsions; reducing the adsorption of PE droplets. For the PE coated with WPM-PB particles (PE-NPB), a flocculation due to protein bridging and protein intramolecular interaction, lead to a network with a fractal dimension of 2.7. For the DS emulsions stabilized by PE-NPB, the interfacial layer thickness of DS emulsion droplets increased with the increase in the concentration of PE as observed by CLSM, whereas the size of DS emulsion droplets decreased. A fractal network consisting of adsorbed PE-NPB on the interfacial layer of DS emulsion was observed by TEM and measured by USANS. These results suggest that both the structure of the interfacial layer and the size of the DS emulsion is dependent on the concentration of the PE used.
Interface structure of 70% fish oil–in–water emulsions stabilized with combinations of sodium caseinate and phosphatidylcholine: use of small angle neutron and X-ray scattering techniques

Marine long chain (LC) omega-3 polyunsaturated fatty acids (PUFAs) have been reported to have numerous beneficial effects on health such as decreasing cardiovascular diseases, improving immune system and mental health. Oil-in-water emulsions have been used as delivery systems to enrich foods with LC omega-3 PUFAs, such as eicosapentaenoic (C20:5n-3), docosahexaenoic (C22:6n-3), DHA acids, in order to increase the intake of these bioactive compounds. However, LC omega-3 PUFAs are highly prone to oxidation, which results in formation of lipid oxidation prod- ucts causing undesired sensory properties as well as loss in nutritional profile. As high fat oil-in-water delivery emulsions are applicable to many food systems and lipid oxidation in emulsions have been claimed to be initiated at the oil-water interface, food researchers and industry are highly interested in understanding the inner dynamics of these systems. This study focused on characterizing interfacial structure of high fat (70%) oil-in-water emulsions emulsified with sodium caseinate (CAS) and phosphatidylcholine (PC). Structure of emulsifiers which are adsorbed on water-oil interface could be best studied by Small Angle Neutron Scattering (SANS) together with Small Angle X-Ray Scattering (SAXS). Interfacial structure was aimed to be characterized by identifying thickness of the interfacial layer and distances between emulsifiers adsorbed at the oil-water interface as well as obtaining information on adsorption behavior of the combined use of emulsifiers in high fat delivery emulsions. In order to study these characteristics, simpler versions of this system was also studied; e.g. different concentrations of CAS in D2O, different concentrations of PC in D2O, 70% oil-in-water emulsions produced only with CAS or only with PC at different concentrations. Contrast matching between oil and water phase as well as contrast variation of water phase were applied. For this reason, scattering length densities and relative concentrations of employed compounds were calculated and hydrogen content was considered to be minimized. From these considerations, it was proposed to have a model system of deuterated water and various mixtures of deuterated and regular hexadecane or only deuterated hexadecane providing maximum contrast between liquids and emulsifiers. Fish oil was also included in some of the measurements in order to compare the results from model systems with the original system.

SANS results have shown that CAS forms aggregates and PC forms multilayers both in water and high fat oil-in-water emulsion. It was found that CAS concentration affected the periodic repeat distance of the PC bilayers. When the mass concentration of CAS increased, the distance between PC bilayers become smaller, this was attributed to the decrease in water amount trapped between PC layers. When the concentration of CAS was fixed, the distance between PC bilayers was the same independently from PC concentration. Moreover, SANS results indicated that the aggregate size was bigger when CAS was in the emulsion interface together with PC compared to the CAS in D2O which might be an indication of the interaction between CAS and PC at the oil-water interface in high fat oil-in-water emulsions.

The memories of liquid triacylglycerols

Crystallization of triacylglycerols (TAG), and other lipids, happens from the liquid into a broad range of polymorphic crystalline forms. Despite the sustained research effort over many years, it is still not possible to predict the crystalline form from first principles. In the case of mixtures, the prediction becomes disproportionately hard, even for just binary mixtures. They exhibit a very large number of seemingly anomalous crystallization behaviours. Among them, the ‘memory effect’ is particularly relevant. Perhaps the liquid structure holds the key to these phenomena. A brief review of the few conceptual models proposed for the organization of TAG molecules in the liquid shows that, until a few years ago, researchers had not been able to offer a solid proposal for the liquid structures. Yet, the hypothetical structures suggested in the literature are often taken for granted.

We present here results from experiments aimed at proposing a structure of the liquid state and its influence on crystallization phenomena that are otherwise hard to explain. Essentially, TAG molecules form clusters due to the difference in attractive forces between two regions of the molecules, the aliphatic chains, and the polar glycerol core. The clustering has been predicted by molecular dynamics and coarse-grained simulations. Pure liquid TAG samples were examined by x-ray scattering at temperatures up to 210°C. Wide angle scattering (WAXS) data are consistent with the liquid phase of aliphatic molecules. Small angle scattering (SAXS) data are similar to those produced by alcohols and fatty acids, whose molecules associate via polar groups. The liquid TAG seem to form “Loose Multimers” of 5 to 9 molecules. The average number of molecules per cluster decreases with temperature and increases with molecular weight.

The ‘memory effect’ is observed when TAG re-crystallize from a liquid, obtained by melting crystalline TAG, and form the same structure that they had as a solid. Differential scanning calorimetry (DSC) experiments were done with tempered pure TAG and TAG mixtures. The materials were melted and held at many [time + temperature] combinations before recrystallizing. Combinations that produced a recrystallization equal to a crystalization from a random liquid defined a time-temperature boundary. Above the boundary, the memory is erased. For pure TAG, the times and temperatures required were shorter and lower than for the mixtures. Some liquid structure of pure triacylglycerols remains after melting, which is disrupted by thermal fluctuations. In blends, it is additionally necessary to homogenize the concentration of domains of different sizes, via molecular diffusion, a much slower process. Ultra-small angle neutron scattering (USANS) experiments were performed using tripalmitin. Data obtained from hydrogenated and deuterated samples were collected at 95°C. Data from the deuterated material were then obtained upon cooling from the melt, and upon melting from the crystalline state. The difference in the scattering patterns indicates the presence of different structural organization in both cases, consistent with the explanation of the ‘memory effect’ due to differences in the clustering of molecules in the liquid state.
Structure formation in oleic acid - sodium oleate based oleogels

The structuring of liquid oil into what are known as oleogels has received considerable attention in recent years. Oleogels have the potential to replace solid fat in food products with healthier unsaturated oils. Various oleo-gelators are known, ranging from large polymers to low molecular weight oleo-gelators (LMWOG). A known combination of LMWOGs is that of oleic acid and sodium oleate. Their ability to gel sunflower oil has already been demonstrated, and gel strength can be tuned by varying the ratio between oleic acid and sodium oleate. The addition of a small amount of water results in stronger gels and suggests hydrogen bonding plays a role in the gelation. [1]

In this presentation we will dive deeper into the relation between the gel strength of oleogels prepared with oleic acid and sodium oleate, and their micro- and nano-structure. Oleogels were prepared with different ratios of the two LMWOGs and using different triglyceride oils. Upon varying the ratio between oleic acid and sodium oleate, changes in both micro- and nano-structure were observed through light microscopy and Small Angle Neutron Scattering (SANS) and Ultra-SANS (USANS). The observed structure ranges from inverse micelles to lamellar crystals, which coexist in some cases. Additional information on the type of crystals was collected using Small Angle X-ray Scattering. In addition to the triglyceride oils, n-hexadecane was used as a solvent analogue. This enabled the use of selective deuteration and provided additional information on the nano-structure. A clear relation between micro- and nano-structure, and the strength of the oleogel was observed.


Static and dynamic multiscale characterisation of micronized fat crystal network formation and disruption by USAXS and rheo-SAXS

Micronization of fat crystals presents an appealing alternative for the conventional melt-cool routes for manufacturing fat based food products. By dispersing micronized fat crystals (MFC) in oil, fat crystallisation and network formation can be decoupled, which can bring significant process simplifications. In order to assess the industrial application scope of these dispersions we carried out a multiscale investigation on MFC network formation and disruption by means of USAXS, rheo-SAXS, confocal Raman imaging and rheo-MRI.

Rheo-SAXS and rheo-MRI showed that upon dispersion in oil, MFC network formation was concomitant with recrystallisation. Oil type, temperature and shear rate collectively determined MFC recrystallisation rate, which inversely correlated with the strength of the resulting weak-link network where crystal aggregates are embedded in a continuous net of crystalline nanoplatelets. US-AXS revealed that the rough surface of MFC nanoplatelets hampers stacking into one-dimensional aggregates (TAGwoods), which explains the high mass fractal dimension of the networks formed in MFC dispersions as compared to those formed by melt-cooling.

Applying shear to matured MFC networks leads to a gradual and irreversible loss of yield stress, as shown by rheo-MRI. Shear did however not affect network fractal dimensions (USAXS) and also did not disrupt micronscale MFC aggregates (confocal Raman imaging). Rheo-SAXS revealed that loss of network strength can be attributed to release of nanoplatelets from the weak-link network, which subsequently align in the shear field and undergo rapid recrystallisation. Our insights in the factors that govern MFC network formation and disruption bear relevance for simplified manufacturing of fat-based food products by effectively turning their design into a colloidal aggregation game.
Heavy food molecules from the National Deuteration Facility for structure-function applications

There have been limited global initiatives in the field of molecular deuteration where the majority of these programs focus on biological deuteration of proteins, while more complex deuterated small molecules like phospholipids, sugars, triglycerides, fatty acids haven’t been widely available to the wider science community. This has limited the experiments that can be performed, and formed a bottleneck for advancing the applications of neutron scattering, mass spectroscopy and NMR.

In this paper we will discuss the recent advancements and the impact of deuteration on the research outcomes achieved by using deuterated molecules produced by the National Deuteration Facility of the Australian Nuclear Science and Technology Organisation. Recent high-impact case studies in the fields of food science and biotechnology will be presented which reveal the exciting and diverse characterisation studies which are now available for the neutron, NMR, IR and mass spectroscopy science communities.

Structural characterization of milk coagulation from 0.1 to 20μm using Ultra-Small Angle Neutron Scattering

Milk is a main component in many food products in a variety of cultures around the world. Research and development in milk over the past five decades has enabled increased shelf-life, better flavor and accurate control over fat size and concentration. Milk is a water-based colloidal system, comprised of soluble proteins, casein micelles (CMs), ions and fat globules (FGs). The addition of a coagulant, the enzyme chymosin, to this system brings about aggregation of the CMs via hydrolysis of the κ-casein molecules which make up the surface of the CMs, effectively trapping the FGs together with some liquid whey, resulting in a complex soft matter system called cheese. The structure and organization of the CMs and FGs in milk have been addressed by many invasive experimental methods probing scales from 0.1nm to 1μm, yielding conclusions via two-dimensional images. Furthermore, the structure of cheese probed by these invasive methods (CLSM, cryo-SEM, etc.) could introduce artifacts to the unperturbed structure. In view of this, it is desirable to utilize a technique that can be non-perturbing and can access the micron-scale structure of cheese.

This technique is ultra-small angle neutron scattering (USANS), which enables us to probe length scales from 0.1μm to 20μm, effectively providing information on the size, surface characteristics, three-dimensional organization, scattering length density and concentration of the main components in milk and cheese. Our USANS studies were carried out on the BT-5 thermal neutron beam port at the NIST Center of Neutron Research, Gaithersburg, Maryland. Scattering results from homogenized milk revealed smooth-surfaced FGs with mean diameters of 540nm, polydispersity of 40% and concentration of 3.28%. Utilizing the contrast matching technique with homogenized milk samples and overlapping the signal with scattering data from skim milk, CMs were found with mean diameters of 120nm, polydispersity of 16% and concentration of 1.5%.

When homogenized milk was coagulated with chymosin for one hour, CMs were found to be organized in one-dimensional aggregates having an average thickness of about two CMs. Correlations of coagulation time with aggregate thickness and surface roughness displayed aggregate sizes doubling (for two hours), then slightly decreasing (for three hours), while the surface became smoother as coagulation time increased. During skim milk coagulation, smooth aggregates of two CM thickness formed quickly (within 1 hour), continuing to double in thickness while becoming rougher (within 2 hours) - possibly attributable to the absence of FGs.

Our results suggest that the USANS technique is a promising method to probe micron-scale structure of commercial milk in natura, as well as milk coagulated via enzymatic activity, since our results pertaining to diameter and concentration are in agreement with those in the literature. Pros and cons of this technique with regard to applications in milk and cheese studies will be highlighted and data analysis methods discussed. A comparison to the USAXS technique will also be offered. Characterization of mean sizes, polydispersity’s and independence in the organization of CMs and FGs, can contribute to optimization in the manufacture of healthy milk-based food products.
Ultra–Small Angle Neutron Scattering investigation of milk coagulation: Data analysis and contrast matching methods

Milk, a water-based colloidal system, is comprised of fat globules (FGs), casein micelles (CMs), soluble proteins and ions. The mechanism of cheese formation from milk, through the induced aggregation of CMs by the addition of chymosin, is a complex soft matter process. This process has been widely characterized using invasive experimental methods (confocal microscopy, electron microscopy, etc.) that have probed milk and cheese at length scales from 0.1nm to 1μm, providing two-dimensional images of the organization and structure of the main components in milk, viz. CMs and FGs. Given that these techniques could impose artifacts on the system, in the present study we utilized ultra-small angle neutron scattering (USANS) to investigate the coagulation of homogenized whole (HW) and skim milk in natura. The USANS instrument probes length scales from 0μm to 20μm, providing information on size of the clusters, surface characteristics, three-dimensional organization, scattering-length density (SLD), polydispersity and volume fractions of the main components in the milk and cheese samples. An advantage of using neutrons is the ability to utilize contrast matching, a technique that varies the scattering-length of the hydrogen-deuterium component in the system to effectively blend certain objects (CMs or FGs) into the background, so as to observe a clean signal from the object of interest. Raw USANS data can be analyzed using a variety of models to describe the micron-structure of the scattering objects. We have implemented two analytical models, Guinier-Porod (GP) and Bimodal Schulz-Spheres (BSS). These models provide us with the most persuasive interpretation of the process of milk coagulation. Through examination of the slopes using the GP model, we found correlations of coagulation time with the CM aggregate thickness and surface roughness, as well as the shape or packing symmetry of the aggregate. Analysis using the BSS model enabled us to identify the polydispersity, volume fraction, SLD, and average radii of FGs and CMs in HW milk. With this model it is possible to decompose the combined scattering effects from two objects (assumed to be spheres) within a liquid medium, while taking the variability in radii of the polydisperse objects into account. Contrast matching confirmed the observed three-phase system by running a series of HW milk samples diluted with different ratios of D2O to H2O using the predicted SLDs and given volume fractions. Blending of the FGs in HW milk (3.25 wt% milk fat) with the background signal proved successful when compared to the skim milk (< 0.5 wt% milk fat) data, as it showed little to no difference (Figure 1). Our findings demonstrate the value of USANS in combination with GP and BSS modeling techniques as analytical tools for the characterization of the micron-structure of commercial milk in natura, as well as coagulated milk obtained via enzymatic action.

AUTHORS
Nukhalu Callaghan-Patrachar 1
Carl Adams 2

CO–AUTHORS
David Pink 1
Alejandro Marangoni 1
Fernanda Peyronel 3

1 University of Guelph
2 St. Francis Xavier University

CORRESPONDING AUTHOR
patrachar8@gmail.com

Contrast Matching with Homogenized Whole Milk
Dynamics of calcium caseinate in H\textsubscript{2}O or D\textsubscript{2}O studied by Quasi-Elastic Neutron Scattering (QENS)

Producing meat analogues using calcium caseinate is an intermediate step to reduce carbon emission and improve animal welfare. Depending on the drying methods of the powder, 30\% w/w spray-dried calcium caseinate (SCaCas) forms pronounced fibrous structure under shear and heat of 50\(^\circ\)C, while roller-dried calcium caseinate (RCaCas) does not [1]. Solvent plays a role in the fibrous structure as well. When SCaCas is prepared with D\textsubscript{2}O, the formation of fibrous structure is not possible while RCaCas shows anisotropy in the final structure [2]. We hypothesize that drying methods and solvent environments affect the hydration behaviour of the protein powders.

To systematically study the dynamics of calcium caseinate, we performed QENS experiments on both SCaCas hydrated with H\textsubscript{2}O (SH) or D\textsubscript{2}O (SD) and RCaCas with H\textsubscript{2}O (RH) or D\textsubscript{2}O (RD). Samples with a hydration level of 0.4 g/g dry powder were measured with an energy resolution of ~17.5 \(\mu\)eV and \(Q\)-range of 0.5-17 \(\text{Å}^{-1}\).

Mean Square Displacements (MSDs) were extracted by fitting the elastic window scans with Gaussian approximation and is shown in figure 1. The ‘dynamical transition’ temperature of the SH is the highest (250K), and its slope above 250K the steepest. Results suggest, for RCaCas, the activation energy for protein dynamics is insensitive to solvent. While for SCaCas, the protein dynamics is the same as RD when hydrated with D\textsubscript{2}O, but more energy is needed to induce dynamic transition when hydrated with H\textsubscript{2}O. Plus, the water dynamics of SH is more active comparing to the rest.

The full energy spectra are fitted with the sum of a Delta function, a Lorentzian function and a linear background. The Full Width Half Maximum (FWHM) of the Lorentzian is fitted with the SingwiSjölander model to describe the motions of water molecules. The translational diffusion coefficient (\(D_t\)) and residence time (\(\tau_0\)), plotted in figure 2, were compared between samples at 293K (room temperature) and 320K (50\(^\circ\)C). The \(D_t\) of both samples increased with increasing temperature, with RH displaying a slightly higher increase (from 0.15 to 0.30 Å\textsuperscript{2}/ps, compared to 0.17 to 0.27 Å\textsuperscript{2}/ps for SH). As for \(\tau_0\), both samples decreased with increasing temperature. In contrast to bulk water, whose \(\tau_0\) is typically 1ps, the water molecules are quite confined to the protein surface, even at elevated temperature. The difference between SH and RH may be attributed to the differences in initial powder morphology and conformational changes in structure at a higher temperature.


Ultra-small angle neutron scattering studies on milk and cheese curd formation

Milk and milk products are essential to global nutrition and the world-wide food industry. We have carried out ultra-small angle neutron scattering (USANS) studies on samples of “as-is” commercial food-grade milk and samples with added rennet, as the first step in cheese formation. Casein protein micelles are present in both skim and whole milk with median diameters of 120 nm with 16% polydispersity. In homogenized milk (1.25% milk fat) the median diameters of fat globules are 0.54 μm with a polydispersity closer to 40%. Despite various structural features implied by microscopy (rough kappa casein molecules, porosity, a membrane layer for fat globules, co-aggregation of micelles and fats) both of these components can be modelled by smooth, independent, uniform spheres. Contrast matching gives scattering length densities (SLD) of 0.20 × 10^{-6} Å^{-2} for fats and 0.89 × 10^{-6} Å^{-2} for casein micelles. A typical protein SLD is twice this value so the micelles are likely quite hydrated. Rennet dramatically increases the scattering at the smallest angles, consistent with the formation of large structures. As the curd making process continues, we observe further aggregation.

AUTHORS
Carl Adams 1
Nukhla Callaghan-Patrachar 2
Fernanda Peyronel 3
David Pink 4
Alejandro Marangoni 2

1 St. Francis Xavier University
2 University of Guelph

CORRESPONDING AUTHOR
cadams@stfx.ca

Structure of artificial casein micelles composed of deuterated β-casein and native κ-casein: A contrast variation SANS and SAXS study

Milk is one of the most important commodities in the world and yet the colloidal protein structure, the casein micelle, that gives rise to many of the unique properties of milk is still not well defined due to its complex and dynamic structure. To gain a detailed picture of the structure of the casein micelle, we produced a world-first model artificial casein micelle comprising recombinant deuterated, phosphorylated, β-casein and native κ-casein, a micelle composition not dissimilar to “human” casein micelles. Using contrast variation SANS and SAXS, a detailed structure of the casein micelle will be presented.

AUTHOR
Jared Raynes 1

CO-AUTHORS
Jitendra Mata 2
Carl Holt 3
John Carver 3

1 CSIRO
2 ANSTO
3 University of Glasgow

CORRESPONDING AUTHOR
jared.raynes@csiro.au
Neutron imaging application in food science on DINGO at OPAL

The new neutron radiography / tomography / imaging instrument DINGO [1] is operational since October 2014 to support research at ANSTO. It is designed for a broad national and international scientific user community and for routine quality control for defense, industrial, cultural heritage and archaeology applications. With experience from materials science and archeology we would like to connect to the field of food science application. DINGO provides a useful tool to give a different insight into objects because of different contrast compared to X-rays and high sensitivity to light elements. Since being operational we gathered experience in various scientific fields, with industrial applications and commercial customers demanding beam time on DINGO. The measured flux (using gold foil) for an L/D of approximately 500 at HB-2 is 5.3 x 10⁷ [n/cm²s] allows us to run neutron tomography experiments in a reasonable time scale from 3 hours to 3 days per tomography, depending on sample composition and resolution. A special feature of DINGO is the in-pile collimator position in front of the main shutter at HB-2. The collimator offers two pinholes with a possible L/D of 500 and 1000. A secondary collimator separates the two beams by blocking one beam and positions another aperture for the other beam. The neutron beam size can be adjusted to the sample size from 25 x 25 mm² to 200 x 200 mm² with a resulting pixel size from 13μm to ~100μm. The whole instrument operates in two different positions, one for high resolution and one for high speed. We would like to present example experiments with potential new applications in food science.

The potential of scattering techniques to investigate the structure and molecular interactions of polysaccharides

Scattering techniques represent an excellent tool for the structural characterization of natural polysaccharides since they involve minimal sample preparation and do not require drying processes which can strongly affect the native structure of hydrated systems. However, the application of this field is still a largely unexploited area due to their specificity and the complexity of data manipulation and interpretation.

In this talk, the application of small angle X-ray and neutron scattering (SANS and SANS) as well as wide angle X-ray scattering (WAXS)/X-ray diffraction (XRD), in combination with complementary methods such as differential scanning calorimetry (DSC), spectroscopy and morphological characterisation, to investigate the structure and molecular interactions of polysaccharides in hydrated systems with different practical applications, such as the following: (i) plant cell wall (PCW) materials, including model hydrogels based on pure cellulose and composites with PCW polysaccharides (arabinogalactan, xylan, mixed linkage glucans and pectins), mature cotton fibres and food-extracted PCWs; (ii) starch-microalgae aqueous blends for the development of glucans and pectins), mature cotton fibres and food-extracted PCWs; including model hydrogels based on pure cellulose and composites characterized by solvent specific extraction of Plantago ovata seed mucilage: AX-A(alkali) and AX-W(water) [1-3]. Both fractions are found to be neutral bottle-brush polysaccharides exclusively comprised of xylose and arabinose with similar molecular weight and linkage composition. Despite compositional similarity, their rheological and structural properties are markedly different. Using a combination of USANS/SANS with rheological characterisation techniques, we have established the hierarchical nature of hydrogel assembly, with pore sizes in the range between 30 and 1000 nm. Using enzymatic cleavage of terminal arabinoses of side chains we induced significant changes in rheological and structural properties of these complex arabinoxylans, which enabled us to corroborate that (i) hydrogen bonding between side chains of neighbouring molecules is a key driver of gel formation; (ii) the strength of hydrogen bonding is motif specific, which explains differences in physical properties between two fractions. It is envisaged that discovery of motif-dependent interactions will open new opportunities for rational design of polysaccharides with targeted and highly tuned physical properties, providing a far-reaching potential for delivering novel tailor-made hydrocolloids for use in foods, pharmaceutical industry, and smart materials.


AUTHORS

Marta Martínez-Sanz ⁱ
Amparo López-Rubio ⁱ
Patricia López-Sánchez ²
María José Fabra ³
Laura G. Gómez-Mascaraque ³
Michael J. Gidley ⁴
Elliot P. Gilbert ⁴

¹ Catedrático Agustín Escardino
² IATA-CSIC
³ RISE
⁴ Teagasc
⁵ University of Queensland
⁶ ANSTO

CORRESPONDING AUTHOR

mmartinez@iata.csic.es
Supramolecular characterisation of starch in rice by NMR, SAXS and XRD

Rice has fed more people over a longer period of time than any other grain [1] and currently provides 85 % of the energy intake to over half the world’s population [2]. This makes it a suitable vehicle to help manage weight and obesity-related illnesses, especially type 2 diabetes and colorectal cancer.

Starch is the major component of rice (~90 %) and its supramolecular structure is known to affect rice digestibility. However, starch is one of the most complex materials found in nature with six known hierarchical levels of structure [3]. In rice, starch granule heterogeneity and its influence on digestibility depends upon genetic makeup, environmental influences (between geographical locations and seasons) and processing (such as milling and cooking).

Multiple levels of starch structure in cooked and raw rice were characterised to better understand how starch structure affects digestibility. Long range crystallinity was characterised by powder X-ray diffraction (XRD). Smaller scale crystalline structure in the form of helix content was assessed by 13C solid-state nuclear magnetic resonance (NMR) spectroscopy. The semi-crystalline lamellar structure of starch in rice was characterised by small angle X-ray scattering (SAXS) [4]. Complementary to SAXS, small angle neutron scattering (SANS) is a powerful technique in the analysis of soft condensed matter [5]. Sample versatility and solvent contrast makes SANS especially attractive in the analysis of granular starches under conditions relevant to food such as cooking [6]. Molecular mobility was also assessed by 1H solid-state NMR (determining the T2 relaxation time of starch in cooked rice).

Multiple levels of starch structure in cooked and raw rice were characterised to better understand how starch structure affects digestibility. Long range crystallinity was characterised by powder X-ray diffraction (XRD). Smaller scale crystalline structure in the form of helix content was assessed by 13C solid-state nuclear magnetic resonance (NMR) spectroscopy. The semi-crystalline lamellar structure of starch in rice was characterised by small angle X-ray scattering (SAXS) [4]. Complementary to SAXS, small angle neutron scattering (SANS) is a powerful technique in the analysis of soft condensed matter [5]. Sample versatility and solvent contrast makes SANS especially attractive in the analysis of granular starches under conditions relevant to food such as cooking [6]. Molecular mobility was also assessed by 1H solid-state NMR (determining the T2 relaxation time of starch in cooked rice). This work aims to characterise the structural features of starch in a range of rice varieties, and to relate this information to rice digestibility. It is expected that the supramolecular structure of starch in rice will have a significant influence on the digestion of rice.


Supramolecular characterisation of starch in rice by NMR, SAXS and XRD

If we could design plant protein structures and tune properties in processed food

Plant proteins from pea, wheat and quinoa during latest years have become attractive alternatives to replace imported soya in various food applications in Europe. Proteins in a form of protein-rich fractions (from pea and wheat), and the whole flour (from quinoa) can be highly attractive ingredients in making pasta, protein-rich snacks and various types of breakfast food. Structure, state of aggregation and morphology of protein components is a key factor determining of their functional properties in these foods and is greatly impacted by the processing method used. Therefore, a better control and understanding is needed on how protein structure and function are related in various protein containing systems of the processed food.

Here, we show few examples on structural-function relationship of the processed pea, wheat and quinoa proteins into pasta-like sheets, textured protein snacks and breakfast food. We studied the protein structure and protein interactions (including components, as dietary fiber) using synchrotron WAXS/SAXS, X-ray scattering tomography, SEM and HPLC techniques. Variation in processing conditions was high and low processing temperature, composition of a blend and a processing method used. The results indicated that pea protein fraction in a blend with pea fiber showed polymerization behaviour that was greatly depended on the protein to fiber ratio (more protein greater polymerization). An increase of dietary fiber in the blend with pea protein resulted into high amounts of unordered structures observed by FTIR and in an increase in the distances of the scattering elements observed by SAXS. The mechanical properties, as strength and extensibility, of the pea protein and fiber blends were the highest for greatest amounts of protein having blends, while the E-modulus was similar for all the studied blends. For wheat protein fraction, gluten, the structure and polymerization of gluten textured snack were influenced by the composition of the blend as was observed by X-ray scattering tomography. Large microstructural differences, mainly due to high processing temperature treatment, were observed in the quinoa extrudates studied by SEM and X-ray scattering tomography.

Here we conclude, that understanding on how to control structure on macro- and nano- levels is a key element when tuning functional properties for the diverse protein systems in food. Once the protein structure is better understood, it would allow better steering of processing and control of final end-use characteristics of the protein rich food products.
Neutron scattering study of lipid sponge-phase nanoparticles as enzyme carriers for food processing

Immobilization of enzymes into different support materials has been widely used as means to control their activity and stability. This has mainly been done in context of bioanalytical, preparative or biomedical purposes. In the present study we investigate two key types of enzymes used in food processing, namely Aspartic protease (34 KDa) and Beta-galactosidase (460 KDa), which are used in processing of dairy products.

Mostly these proteins are delivered into the process as solutions with a considerable amount of preservatives and still with limited shelf-life and limited control of the enzyme activity. Here we have used lipid liquid crystalline phases as enzyme carriers, based on their established capability for drug delivery, protein encapsulation or crystallization. They can form a wide range of self-assembled structures with aqueous cavities of nano-scale dimensions. Reverse cubic or hexagonal lipid aqueous phase can be used to entrap smaller biomolecules such as proteins, here, we will present a novel lipid system able to form highly swollen sponge phases (L3), with aqueous pores up to 13 nm of diameter.

We will show that this structure is preserved even if in excess aqueous solution, where they form sponge-like nanoparticles (L3 NPs) in which the two enzymes are included. The structure and composition of the particles was revealed by combined measurements using small angle neutron scattering (SANS), light scattering, cryo-TEM, size exclusion chromatography and Raman spectroscopy. The SANS results reveal differences in the L3 NPs with and without enzyme. To reveal the nature of the interaction between the enzymes and the lipid matrix, we further studied the adsorption of both proteins on the lipid layers formed by the L3 NPs. These data reveal partial penetration of the enzymes in the lipid bilayers. The results of this study will be discussed in terms of the ability of these nanoparticles to encapsulate and release of the proteins in the lipid matrix.

AUTHORS
Maria Valdeperas Badell 1
Najet Mahmoudi 2
Susana C.M. Teixeira 3
Welbourn Welbourn 2
Maximilian Skoda 3
Gunnar K. Pålsson 3
Justas Barauskas 3
Tommy Nylander 1

1 Physical Chemistry and Nanoclund, Lund University
2 ISIS, STFC, Rutherford Appleton Laboratory
3 NIST Center for Neutron Research and Dep. of Chemical & Biomolecular Eng., University of Delaware
4 Department of Physics, Uppsala University and Institut Laue Langevin
5 Camurus AB and Biomedical Science, Malmö University

CORRESPONDING AUTHOR
Tommy.nylander@fkem1.lu.se

A combined SANS and USANS study to investigate the structure of solid lipid nanoparticles

Solid lipid nanoparticles (SLNs) have been extensively investigated as drug carrier systems since their inception in the 1990s. These are emulsions when prepared at high temperature and crystallize predominantly into SLNs upon cooling. The details of their ultrastructure are poorly defined. Previously, our group reported a novel microwave-assisted microemulsion-based technique to prepare SLNs with radii of approximately 150 nm.

To understand the detailed internal structure of these SLNs, contrast variation ultra-small angle neutron scattering (USANS) and small angle neutron scattering (SANS) experiments were conducted on suspensions of hydrogenated stearic acid SLNs in D2O. Together, SANS and USANS gave a combined Q range of 0.000047 to 0.6 Å^-1 (corresponding to a size range of 1 nm - 15 μm). The extended Q range used in this study allowed an extensive study of the hierarchical structure of SLNs.

The combined data are consistent with the SLNs having an oblate structure at the microscale level, intermediate between rods and lamellae, with roughened surfaces. At the nanoscale level, the results were consistent with the SLNs having an ellipsoidal shape intermediate between spheres and rods, with a crossover from mass fractals to surface fractals. The elucidation of this structure is particularly important given that the structure influences the stability and drug release properties of the nanoparticles. These results will assist in the development of systems with desired shape and properties.

AUTHOR
Rohan Shah 1

CO-AUTHORS
Jitendra Mata 2
Gary Bryant 3
Daniel Eldridge 4
Ian Harding 5
Enzo Palombo 1
Alexander Ife 1
Snehal Jadhav 1
Avinash Karpe 1

1 Swinburne University of Technology
2 ANSTO
3 Centre for Molecular and Nanoscale Physics, School of Applied Sciences, RMIT University
4 Swinburne University
5 Deakin University
6 CSIRO

CORRESPONDING AUTHOR
rshah@swin.edu.au
Neutrons for industry – ACNS ILO, ANSTO – Access, services and case studies

The Australian Centre for Neutron Scattering at ANSTO is a multidisciplinary international centre of excellence, specialising in applying X-ray and neutron scattering techniques to the study of matter in various physical states: solid, liquid and gas. Over time, we’ve built an exceptional body of skills, experience and technical expertise, which we now offer to support industrial research and development.

The Australian Centre for Neutron Scattering Industrial Liaison Office (ACNS-ILO) was established in April 2014 to manage technology transfer and promote the use of our facilities in applied industrial research.

Neutron characterisation can provide direct impact into the optimization of modern manufacturing processes, improved product reliability, enhanced design performance, reduced production cost, and extended life of significant engineering assets.

This presentation will showcase our flexibility in access and services, as well as highlight our recent achievements in helping industry across many sectors.

Structural investigation on nanostructured lipid carriers for fish oil by small angle scattering

Fortification of foods with essential functional lipids such as ω-3 polyunsaturated fatty acids (PUFAs), β-carotene and vitamin A are of key importance for health and well-being. ω-3 PUFAs are essential for the human diet to support a good brain and cardiovascular health. Furthermore, they lower the risk of other diseases such as type 2 diabetes and inflammatory disorders. Vitamin A and its precursor β-carotene are important for growth and development, good vision and immune functions. Integrating such chemically unstable compounds into food products and delivering them into the human body requires encapsulating them into a carrier system. This improves their water-dispersibility and protects them from lipid oxidation.

Recent studies [1-3] show that lipid oxidation of ω-3 PUFAs reduces significantly when incorporated into a solid tristearin matrix, a so-called nanostructured lipid carrier, when sufficient amounts of tristearin is added to the samples and lecithin with a high phase transition temperature and bile salts or Quillaja saponins are used as emulsifiers. From this observation it is anticipated that during the cooling process the emulsifier molecules at the lipid-water interface promote a co-crystallization of the lecithin chains with the tristearin via heterogeneous interfacial nucleation. The crystallized tristearin limits the mass transport of reactive agents which reduces lipid oxidation of the encapsulated fish oil.

However, the structural composition of such nanostructured lipid carrier particles is still unclear. Does the crystallized tristearin form a shell around a single fish oil core or is the fish oil embedded as smaller islands in the tristearin particle matrix?

Typically, these lipid particles are about 150 nm in size as measured by dynamic light scattering. Based on the sample composition (fish oil : tristearin ratios between 60:40 and 20:80) and assuming a spherical core-shell structure, we can expect thicknesses between 10 and 30 nm for a tristearin shell [3]. Thus, small-angle X-ray and neutron scattering (SAXS, SANS) and wide-angle X-ray scattering are the methods of choice to study the morphology of the particles and especially their internal structure on atomic to colloidal length scales.

In SAXS and SANS experiments we will study samples with different fish oil : tristearin ratios. Using mixtures of water and deuterated water for the dispersion medium as well as mixtures of tristearin and its fully deuterated analogue, three different neutron scattering contrasts are realized for each sample. The different contrasts allow to highlight the spatial distribution of both fish oil and tris-tearain alone and as a combination in the lipid particles.

After an introduction to the subject, the presentation will show first analysis results of the SAXS and SANS measurements with regard to the structural composition of the nanostructured lipid carriers containing fish oil.

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