NEUTRON SCATTERING: A NATURAL TOOL FOR FOOD SCIENCE AND TECHNOLOGY RESEARCH

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ABSTRACT

Neutron scattering is a powerful tool for the study of soft condensed matter. The use of neutron techniques in combination with traditional characterisation techniques used in food science can provide a unique insight into novel food materials, providing the knowledge to develop new formulations. As these methods have traditionally been poorly utilised in food science research, this paper highlights the potential of neutron scattering techniques in food research and provides some recent examples in its application across food components with an outlook of some potentially interesting applications.

INTRODUCTION

Global trends and consumer demands towards food with increased functionality have driven food industries to develop increasingly complex food systems from sophisticated formulations. To understand and control this increased complexity, interdisciplinary scientific approaches are required (Ubbink & Mezzenga, 2006). Specifically, an improved knowledge about how food components are structured and interact with each other enables the precise manipulation of food molecules for rational design (Sanguansri & Augustin, 2006).

A high proportion of both natural food products, such as milk, and newly developed food structures, are hierarchical in nature. For example, they may contain colloidal structure-building elements, which consist of nano-sized molecules self-assembled into particles or at interfaces. To establish the connection between these structures and their functionality, a shift of focus in food research is needed from macroscopic properties to those on meso- and nano-scales, as these subsequently control the hierarchical structures in food and food functionality. Moreover, tools to investigate both structure and dynamics over broad size and timescales are required.

The potential of nanoscience and nanotechnology in the food industry is widely recognized. In 2000, Kraft Foods established a

NanoteK Research Consortium of 15 universities and national research laboratories to conduct research in nanotechnology for potential food applications (Watkins, 2003). Nestlé have supported research using neutron scattering for some time as well as Unilever and NIZO (De Campo, Yaghmur, Garti, Leser, Folmer & Glatter, 2004; Bouwman, Krouglov, Plomp, Grigoriev, Kraan & Rekveldt, 2004; Bot, Duval, Duiff & Bouwman, 2006; Tromp & Bouwman, 2006). The interactions and assembly behaviour of food components in the nano-range, which determine the microstructure, are known to influence food structure, rheology and functional properties at the macroscopic scale. Among other applications, nanotechnologies are expected to provide breakthrough improvements in controlled delivery. With the significant advances being made in experimental and theoretical approaches to soft condensed matter physics, a deeper understanding of the nature, behaviour and structure-function relationships in foods has been made possible (Mezzenga, Schurtenberger, Burbidge & Michel, 2005).

Neutron scattering is a largely untapped discipline that may be added to the armoury of complementary methods for materials characterisation (Michel & Sagalowicz, 2008). Such studies yield information on the structure and dynamics of the materials,

constituting an important structural tool for the study of soft condensed matter.

While nuclear science and food may not seem to be obvious partners, we will highlight here some of the work that has been carried out using neutron methods on food-based systems and will provide an outlook as to how the range of neutron scattering methods available can potentially be used to gain unique information.

PRINCIPLES OF NEUTRON SCATTERING

Neutron scattering refers to a family of techniques in which neutrons are used as probes to determine structural and dynamic properties of materials by measuring their change in direction and energy after interacting with a sample. An excellent introduction to the basics of neutron scattering has been prepared by Pynn (Pynn, 2000). Light and X-ray scattering techniques will be familiar to many researchers in food science but the origins of the scattering of neutrons from a material are physically similar. The scattering of visible light results from differences in polarisability, for X-rays from differences in the electronic structure of the atom, while for neutron scattering, it depends on nuclear structure of the atom. All of these sources of radiation can be understood as having both wave-like and particulate

characteristics; a comparison of light, X-ray and neutron scattering can be found in Lindner & Zemb (1991).

While the number of protons in an atomic nucleus defines the elemental type, it is the number of neutrons that defines the elemental isotope. Since neutrons are scattered by the atomic nucleus, this means that the scattering from different isotopes can differ significantly. The classic example of this is between hydrogen (one proton in nucleus) and its heavier isotope, deuterium (one proton and one neutron). In this case, the extent of neutron scattering, defined by a length whose magnitude effectively defines the size of the nucleus, is -0.3742×10^{-12} cm for hydrogen and 0.6671×10^{-12} cm for deuterium (Table 1); this length also represents the spatial extent of a pseudo-potential thus the negative sign for hydrogen is associated with an effective attractive potential. This particular difference in scattering length between hydrogen and deuterium is extremely valuable for the study of hydrogen-containing materials and forms the basis of a method known as contrast variation that will be discussed in more detail below. Whereas the extent of scattering by a neutron is determined by the nuclear structure, for X-ray radiation, it is determined by the electronic structure of the target atom. Since the number of protons in an atom is equal to the number of electrons, the X-ray scattering intensity

increases linearly with atomic number. Consequently, it is the heavier elements in a material that will dominate the X-ray scattering. It is also possible to define a scattering cross-section, derived from the scattering length, which is a measure of the effective surface area of the target nucleus presented to the incident neutron; this parameter is proportional to the probability that a scattering event will occur. The difference between the neutron and X-ray scattering lengths and associated cross-sections for biologically-relevant elements is shown in Table 1 (Sears, 1992).

INSERT TABLE 1 ABOUT HERE

SELECTION OF NEUTRON SCATTERING METHOD

One can broadly classify neutron scattering techniques as either elastic or inelastic. Elastic neutron scattering defines a process in which the energy, or equivalently, wavelength of the neutron does not change as a result of the scattering event with nuclei in the target sample. Neutron techniques in this category include small-angle scattering (SANS), ultra-SANS, reflectometry and powder diffraction. These techniques provide information about structure ranging from the sub-Angstrom ($< 10^{-10}$ m) to supra-micron size range ($> 10^{-5}$ m) (Figure 1). This could be the ordered structure of a fibre (neutron

diffraction), the structure of a casein micelle (SANS), the conformation of a protein at an interface (neutron reflectometry) or the arrangement of droplets in an emulsion (ultra-SANS). A more recent technique to emerge to study food-based systems is spin echo small-angle neutron scattering (SESANS) that utilises one of the fundamental properties of a neutron known as spin. The accessible spatial range using elastic neutron scattering techniques is shown in Figure 1. To assist the reader, the hierarchical structure of starch is also shown in addition to complementary characterisation methods.

Inelastic neutron scattering involves an energy change as a result of a scattering event in which the neutron may lose or gain energy by imparting energy from or to the sample respectively (e.g. via a diffusional process). These techniques provide information on dynamics across a broad temporal range with vibrational spectroscopy (~10^{-14} seconds) through to quasielastic neutron scattering (~10^{-13} - ~10^{-9} s) and spin echo spectroscopy (down to ~10^{-7} s). These techniques can provide simultaneous spatial information if angular dependent information is collected. Detailed descriptions of the range of neutron scattering techniques and their broad application may be found in the work from (Byron & Gilbert, 2000).

INSERT FIGURE 1 ABOUT HERE

There is enormous potential in utilising neutron scattering to determine structural and or dynamic information; the challenge is selection of the correct technique and the optimised design of the experiment to yield the desired information. This may be achieved through a discussion with the local 'instrument scientist', a person responsible for enabling visiting experimenters to conduct the most appropriate experiment. A listing of these is usually found on the website of all reactor and spallation-based nuclear facilities.

WHY NEUTRON SCATTERING?

To investigate the properties and their effects on the final characteristics of the food product, it is of outmost importance to maintain the environment as close as possible to the original conditions. In this sense, neutron scattering possesses particularly attractive attributes. Neutron scattering constitutes a non-invasive, non-disruptive technique which enables the study of a sample under realistic conditions including partial hydration and solutions. A scattering event may occur when a neutron (with dimension ~10^{-15}m) interacts with the atomic nucleus of the sample which is

approximately ten times larger. To use a particle analogy and on a length-scale easier to envisage, if a neutron were the size of a pea, the nucleus would be the size of a tennis ball. In a typical material, these tennis balls would be separated by approximately 1 km! As far as a neutron is concerned, materials consist mostly of empty space and neutrons are therefore a highly penetrating form of radiation. As a result, neutron scattering provides bulk information with the scattering representative of the whole sample (as compared, for example, to scanning electron microscopy where only local information is obtained). In addition, neutron scattering can also be applied to study materials contained within thick and complicated sample environments so that one may measure the structural changes of a material during a process. For example, one may study the influence of shear on the formation of micelles in complex fluids by transmitting a neutron beam through a Couette shear cell (Porcar, Hamilton, Butler & Warr, 2004) or the onset of dynamics in hydrated proteins by measuring the quasielastic neutron scattering after passing a neutron beam through a cryostat (Paciaroni, Cinelli, Cornicchi, De Francesco & Onori, 2005). One can mimic different processes and carry out real time-resolved experiments studying the structural changes that occur as a consequence.

The technique of contrast variation (or contrast matching) relies on the different scattering lengths of hydrogen and deuterium. One may define a corresponding scattering length density which represents a molecular property for which the individual atomic scattering lengths are summed and normalised to a physical density. This yields an overall scattering length density for H_2O that is negative (-0.56×10^{10} cm^{-2}) and that of D_2O is positive (6.38×10^{10} cm^{-2}). Thus, through the preparation of mixtures of H_2O and D_2O, particular components may be strategically contrast matched so that they effectively become transparent to neutrons.

Figure 2 shows the scattering length density for water and various biological macromolecules as a function of the deuterium concentration (Hammouda, 2008, adapted from Jacrot, 1976). The range of scattering length density that may be achieved through merely mixing normal and heavy water means that a selected biological component in a multi-component system can be contrast matched so that it has no contribution to the overall scattering. For example, a protein can be studied at an air-water interface in which the water has a composition of 8% D_2O and 92% H_2O. This composition is such that it yields a water phase that perfectly matches the scattering length density of air (the so-called contrast null condition) and is transparent to neutrons. A neutron reflectivity

experiment of such a system would reveal information only on the structure of the protein at the interface including surface conformation, orientation, adsorbed layer density and thickness (Lu, Zhao & Yaseen, 2007). Contrast variation may be achieved based on inherent differences in scattering as a result of chemical composition (as shown in Figure 2) or arise from strategic selective deuteration e.g. replacement of hydrogen with deuterium in a fatty acid.

Neutrons can be scattered coherently or incoherently. Coherent scattering arises from correlations between the neutrons scattered from different nuclei in the sample and yields information on structure. Therefore, the advantages discussed so far relate to coherent scattering and the associated coherent scattering cross-section. Incoherent scattering is spatially isotropic and arises from correlations between the same nuclei at time zero and a later time, t. Incoherent scattering therefore provides details on dynamics. The incoherent scattering cross-section for most isotopes occurring in biological materials is either zero or close to zero whereas hydrogen has a value of 80 barns (one barn = $10^{-24}cm^2$) (Table 1). Thus, the incoherent scattering signal is extremely sensitive to the motion of hydrogen.

Unlike more commonly available characterisation techniques, neutron scattering probably represents the epitome of non-portable

methods. To conduct a neutron scattering study, one needs to visit the source of neutrons; this is either a research reactor (e.g. OPAL in Australia) or a spallation source (e.g. SNS in the United States). Moreover, to conduct experiments at one of these facilities, a peer-reviewed and thus competitive beam time allocation is required. While the high penetration of neutrons is valuable for studying bulk properties, it naturally also means that neutrons have only a weak interaction with the material under study; another relevant consideration is therefore flux. The most intense source of neutrons in the world currently for SANS (~10^8 neutrons $cm^{-2}s^{-1}$) is small when compared to a 1 mW red laser of ~10^{17} photons $cm^{-2}s^{-1}$ (Higgins & Benoit, 1994). Neutron sources are therefore relatively “dim” and neutron beams have to be typically large (perhaps tens of millimetres or more). As a consequence, similarly 'large' samples are required to minimise the data collection time and attempts must be made to maximise the scattering contrast. While those working in the food arena are unlikely to be concerned that 'large' here describes gram quantities, if one is studying a precious, well-defined and perhaps deuterated protein in solution, this may be prohibitively expensive. On this last comment, it is worthy of note that several neutron scattering centres now have deuteration laboratories to enable tailored deuteration of biomolecules (Teixeira et al., 2008). Finally,

neutron scattering techniques yield non-visual information and mathematic models, perhaps even molecular simulations, are often required to interpret the scattering data. Since these models are inherently based on some knowledge of the system (e.g. chemical composition, physical density, hydrodynamic radius, X-ray crystal structure), it is essential that the information obtained from other techniques can be incorporated to generate a physically and chemically robust and meaningful model so as to minimise the semi-infinite number of possible solutions to the scattering data.

SMALL-ANGLE NEUTRON SCATTERING (SANS)

Small angle neutron scattering is a technique able to probe structures over a size range from approximately 1 nm to several hundreds of nm. It is unarguably the most popular neutron technique for the study of food systems and, thus, the one most covered in the present review. Its applications extend from the elucidation of the quarternary structure of a protein, the conformation of a polysaccharide chain and the lamellar structure in granular starches. There are a number of excellent review articles on small-angle neutron scattering (e.g. Jacrot, 1976; May, 2002; Wignall, 1993) that describe the broad application of the method and experimental geometry. This technique is complementary to SAXS (small angle X-

ray scattering), providing the advantage of the contrast variation enabling structural features of different components to be distinguished via contrast matching. This can permit, for example, the analysis of hydrophobic and hydrophilic regions within proteins or the structure of detergents or lipids complexed within solubilised membrane proteins. This approach also makes this technique especially attractive for the study of encapsulating matrices.

INSERT FIGURE 2 ABOUT HERE

i) Studies of starch granules and resistant starch

The molecular structure of the starch granule has been revealed using both SAXS and SANS (Donald, Kato, Perry & Waigh, 2001). Starch granules are considered to be semicrystalline structures with a lamellar arrangement of the two main constituent biopolymers (amylose and amylopectin). SAXS patterns from hydrated native starches show a broad scattering peak, from which the average thickness of the lamellar repeat unit (crystalline plus amorphous region) can be calculated (Jenkins & Donald, 1996). SANS provides the additional ability to quantify the distribution of water within the granule so that comparisons can be made both between different species and processes. Donald and co-workers (2001) used SANS

to assess the validity of cluster model for the starch structure - consisting on 3 regions, i.e., semicrystalline stacks containing alternating crystalline and amorphous lamellae, embedded in a matrix of amorphous material - and to follow the gelatinisation behaviour of a range of starches allowing the location of water during the swelling of the granule before the melting transition of the materials (Jenkins & Donald, 1998).

Resistant starch (RS) is a fraction of starch that is not digested in the small intestine of healthy individuals and arrives to the colon where it is fermented into short-chain fatty acids. The latter molecules are beneficial for the correct functioning of the bowel and implicated in disease prevention (Topping & Clifton, 2001). Recently, we have performed, to the best of our knowledge, the first SANS studies on the resistant fraction of a processed high amylose starch. Figure 3 shows the neutron patterns obtained at 4 different solvent conditions (varying the amount of D_2O/H_2O), together with the SAXS.

INSERT FIGURE 3 ABOUT HERE

The five scattering patterns have been simultaneously fitted to a 6 parameter model, including a power law describing the low *q* region and a term describing a two phase non-particulate system that has

previously been observed to properly describe the scattering pattern of resistant starch (Lopez-Rubio, Htoon and Gilbert, 2007). The latter term incorporates parameters that yield the degree of crystallinity, the characteristic dimension and the scattering contrast between the crystalline and amorphous phases. From the fits, it was possible to determine that the contrast match point occurs for a solvent containing 58.6% D_2O, very similar to that of granular starch (Jenkins et al., 1996), indicating that the scattering length differences of amorphous and crystalline phases are identical in native starch and its resistant starch fractions.

ii) Wine stability and structure of Pastis

Physicochemical interactions of polyphenols with polysaccharides and proteins take a primary role in wine stability, clarification and taste. Tannins, for example, are completely soluble in alcohol and form particles only when water is present. Zanchi *et al.* (2007) prepared a model wine (ethanol volume fraction of 12%) composed of tannins that had been extracted from grape seeds yielding chains of 11 flavan-3-ol monomer units (DP11), in deuterated water and alcohol. SANS reveals only small DP11 tannin polymers in solution down to a level of 68 per cent of alcohol; below this alcohol concentration, a sudden increase in scattering is observed

corresponding to the formation of a colloidal state via a nucleation and growth mechanism. Interestingly, by producing two samples from different routes yielding the same chemical composition, the authors found that the size and internal structure of the tannin particles depends sensitively on how the sample was prepared (Zanchi et al., 2007).

Pastis, the aniseed-based beverage, has also been structurally characterized by SANS. The principal aromatic component of this drink is trans-anethol (1-methoxy-4-(1-propenyl)benzene), a compound which is soluble in ethanol but essentially insoluble in water. Upon addition of water, a spontaneous formation of an emulsion occurs with droplets of the order of a micron. It is worthy of note that the micron-dimension of the droplets means that SANS is sensitive only to the interface of the droplet with respect to the continuous phase and that SESANS or USANS might be considered to be more appropriate for detailed droplet characterisation. However, associated decreases in interfacial scattering with increasing droplet radius and, therefore, decreasing surface area come out directly from the scattering data. SANS experiments indicated that the size of the droplets depends on the anethol/ethanol volume ratio and grows with time and temperature (Grillo, 2003).

iii) Protein structure

The understanding of protein folding remains one of the major goals of contemporary structural biology. This requires detailed characterization of both folded and unfolded states. It provides a direct measurement of the radius of gyration of a molecule and thus, is very sensitive to the molecule's compactness (a key parameter in characterising the degree of denaturation of a protein) and a description of overall shape of a macromolecule (Trewhella, 1997; Svergun & Koch, 2003).

Amongst food proteins, caseins from milk have been widely studied using SANS, not only in the unfolded state (Aschi, Gharbi, Daoud, Douillard & Calmettes, 2007) but also in monomeric state below their critical micelle concentration (Thurn, Burchard & Niki, 1987; de Kruif, Tuinier, Holt, Timmins & Rollema, 2002) and within the micelle substructure (Holt, de Kruif, Tuinier & Timmins, 2003). Based on previous SANS results from calcium phosphate nanoclusters prepared in the laboratory (Holt, Timmins, Errington & Leaver, 1998), a model for the casein micelle substructure has been proposed consisting on a more or less homogeneous protein matrix containing a disordered array of calcium phosphate particles. These conclusions were made possible because the calcium phosphate and casein components have different neutron scattering length densities

enabling the contribution of the components to the overall scattering to be separated.

SANS has also been used to follow protein crystallization and the influence of salt concentration (Chondakar, Aswal, Kohlbrecher, Hassan & Wagh, 2007), to investigate the structural stability of proteins under pressure conditions (Ortore, Spinozzi, Carsughi, Mariani, Bonetti & Onori, 2006) and to ascertain the structure of protein-polysaccharide (Singh, Aswal, & Bohidar, 2007) and protein-surfactant complexes (Cosgrove, White, Zarbakhsh, Heenan & Howe, 1995) as well as the influence of water on soy glycinin powders (Kealley, Elcombe, Wuhrer & Gilbert, 2008).

iv) Nanoparticles and other delivery systems for controlled release

Controlled release has been widely exploited within the drug industry but much less so by the food industry (Bunjes and Unruh, 2007). This is likely to be an area of significant growth in food science due to the emerging success of functional foods (Sagalowicz, Leser, Watzke & Michel, 2006). Proteins, lipids and carbohydrates can be used as matrixes for encapsulation and controlled release (Ubbink & Kruger, 2006). Protection is needed for many bioactives as they are generally unstable and interact with oxygen or with other food components in the food matrix. For delivery systems, detailed characterisation is a

major part of the research and development work, in order to ensure the generation of systems with desirable properties. SANS is the perfect technique for the characterisation of controlled delivery systems enabling the size and shape of nanoparticles to be obtained (Aswal, 2003; Bolzinger-Thevenin, Grossiord & Poelman, 1999; Cabane, Blanchon & Neves, 2006; Chodankar, Aswal, Hassan & Wagh, 2007), the evolution of the nanoparticles' structure during ingredient loading (Dave, Gao, Schultz & Co, 2007), or as a consequence of different processing methods (Ghosh, Cramp & Coupland, 2006; Koh & Saunders, 2005; Cabane et al., 2006; Mendes & Menon, 1997), providing evidence for the recombination of nanoparticles (Cabane et al., 2006) and the interactions between the matrix and encapsulated substance (Gerelli et al., 2008; Rodgers, Holden, Knott, Finnie, Bartlett & Foster, 2005). Another advantage of using neutrons in these systems is the ability to suppress selectively the scattering from either component by adjusting their scattering length densities relative to the solvent (Cosgrove et al., 1995). In the case of core-shell assemblies of nanoparticles, if the cores are selectively deuterated, then it is possible to make them transparent to neutrons by adjusting the scattering length density of the aqueous dispersion medium through its H_2O to D_2O ratio. This is shown schematically in Figure 4 and is equally possible for non-polar

solvents using, for example, normal and deuterated forms of hexadecane. Parameters such as the thickness of the core and shell or the homogeneity of the internal structure can be extracted by fitting the SANS data to a core-shell model (Riley et al., 2003).

INSERT FIGURE 4 ABOUT HERE

v) Polymers for food packaging

SANS is a powerful technique for studying the nano-domain structures of polymer systems, fillers and particles. Polymer-based nanocomposites represent an area of increased interest due, for example, to the benefits arising from the addition of clays to polymers for food packaging such as reinforcement of the structure and reduced gas permeability (Yoonessi, Toghiani, Daulton, Lin & Pittman, 2005). For instance, montmorillonite dispersions, at low clay contents, show a significant improvement in mechanical properties, heat distortion temperature and gas permeability. Neutron scattering is a well established technique to investigate clay dispersions and their interaction with polymers (Guyard, Persello, Boisvert & Cabane, 2006). Again, via partial deuteration of the solvent, the contrast between the two system components can be varied. As a result, neutron scattering can reveal information not available from X-ray

scattering but which has been used more extensively to date. SANS can be used to study the effect of clay on the conformation of the polymer chains, the degree of delamination and even the number of individual platelets per tactoid by fitting SANS data (Yoonessi et al., 2005). There are fitting models available describing the scattering data from clay platelets distributed as individual platelets and/or tactoids in a matrix which can be either a polymer or a solvent (Hermes, Frielinghaus, Pyckhout-Hintzen & Richter, 2006).

vi) Lipid metabolism/digestion

SANS has also been used to shed light on the physiology of lipid solubilisation in bile and on the digestion process of the bile-emulsified oil droplets (Lopez, Samseth, Mortensen, Rosenqvist & Rouch, 1996; Pignol et al., 2000). It is well-known that the extent of fat emulsification affects the activity of digestive lipases in vitro and may govern digestion and absorption of dietary fat (Armand et al., 1999). The morphologies of various conjugated bile salt-fatty lipid systems have been extensively studied by Hjelm and co-workers, who found sufficient similarities to suggest a common mode of self-assembly (Hjelm, Schteingart, Hofmann & Thiyagarajan, 2000).

vii) Flow behaviour

Tomato ketchup and sauces exhibit thixotropic behaviour or shear-thinning with their viscosity dropping dramatically when stirred or shaken. Such properties derive from long-chain molecules in the systems that obstruct the movement of fluid as a result of network formation, possibly via weak attractive interactions or entanglement. Since neutrons are a highly penetrating form of radiation, a scattering measurement can be conducted in real-time of a complex fluid under flow. Indeed, the SANS of the fluid under shear can be measured simultaneously with the shear viscosity. In this way, the viscosity - a bulk property - may be related to the structure and orientation of network strands - a molecular property (Förster, Konrad & Lindner, 2005). Herle, Kohlbrecher, Pfister, Fischer and Windhab (2007) have used this so-called rheo-SANS set-up to investigate vorticity bands in a worm-like micelle solution. The kinetics of shear-induced relaxation may also be controlled through modification of the system viscosity, for example, through the addition of sugars (Porcar, Hamilton, Butler & Warr, 2003).

viii) Emulsions

Emulsions are ubiquitous in the food arena such as mayonnaise, salad dressing, milk and Pastis (discussed above); mechanisms by which shelf-life may be extended or formulations may be improved

(for example, fat reduction or improved mouthfeel) may be achieved through control of a wide range of factors including droplet size, charge interactions and emulsifier purity. Despite being a most suitable technique for studying emulsions, SANS has not been widely utilised. One of the few examples relates to the structure of high internal phase water-in-oil emulsions in which a series of model emulsion (and associated microemulsion) systems were prepared making use of contrast variation in both the aqueous and oil phases. (Reynolds, Gilbert & White, 2001). Simultaneously modelling of multiple contrast data sets yielded detailed information on the distribution of surfactant at the oil-water interface, the morphology of the micellar fraction present within the continuous oil phase, in addition to the area occupied per molecule. Their data has also been complemented with neutron reflectometry (Reynolds et al., 2003)

ix) Microemulsions

Dispersions of surfactants in water find multiple applications in food, cosmetic and pharmacological products (Mezzenga et al., 2005). Many commercial surfactants are mixtures whose behaviour can be substantially different from that of the individual pure components in addition to driving different self-assembled nanostructures which can be directly studied by SANS. The particles can be of different shapes

and sizes (spherical or ellipsoidal, cylindrical, disk-like, membrane or vesicle). Microemulsions, comprising surfactant, oil and water have enormous potential in the development of functional foods taking advantage of their self-assembled microstructure and thermodynamic stability (De Campo et al., 2004). Another attractive aspect is their ability to solubilize large amounts of lipophilic and hydrophilic food additives. The evolution of the microemulsion structure as a function of surfactant concentration or ingredient loading can be followed by scattering (Dave et al., 2007). For instance, it was observed that addition of salts in surfactant micellar solutions leads to the formation of more than one type of micelle (Aswal, 2003). Moreover neutron scattering can be used to follow the freeze and pressure destabilization of microemulsions to simulate a food process (Ghosh et al., 2006), to provide insight to the mechanism for temperature-induced emulsion gelation (Koh et al, 2005), to check for vesicle stability during freeze-drying and re-hydrating (Cabane et al., 2006), or to study how the structure changes as a consequence of shear forces (Mendes et al., 1997).

OTHER ELASTIC SCATTERING METHODS

I) SPIN ECHO SMALL-ANGLE NEUTRON SCATTERING

A much more recent method that has found application in food structure determination is spin echo SANS (SESANS), a technique that is complementary to SANS being able to measure features larger than 100nm in real-space (Bouwman et al., 2004). The clearest application of this technique in food research involves the characterisation of emulsion particles, (Krouglov et al., 2003). SESANS can also be used to study anisotropic samples (like polymer fibres) provided that one can rotate the sample, and the processes occurring in the preparation of dairy products, including yogurt and cheese, may be followed with the technique (Bouwman et al., 2004; Bot et al., 2006; Tromp et al., 2006). The latter have been studied with support from both NIZO and Unilever.

ii) NEUTRON REFLECTOMETRY

Neutron reflectometry can provide structural information over a similar size range to SANS where the system under study is layered or located at an interface (Penfold et al., 1997). As the name suggests, the technique involves reflecting a neutron beam from the surface and measuring the intensity of the reflected beam as a function of angle of incidence. When the incident and reflected angles are identical - the specular condition - the reflectivity provides a one-dimensional depth profile perpendicular to the interface. Off-

specular reflectivity - the case where the angles differ - yields additional information about in-plane structure although interpretation is more complex.

Neutron reflection is capable of giving structural information about pure and mixed layers simultaneously with information about its composition via contrast difference. Neutron reflectometry has demonstrated that the air-water interface has a destabilizing effect on the structure of β-lactoglobulin and, thus, a lower energy is needed to unfold the protein when compared to that in bulk solution despite little distortion being caused to the globular framework, (Perriman, Henderson, Holt & White, 2007). This technique has been widely used to study protein adsorption, in addition to protein/surfactant and protein/polysaccharide interactions at interfaces including milk proteins due to the application of the latter in stabilizing foams and emulsions (Cooke, Dong, Thomas, Howe, Simister & Penfold, 2000; van Well & Brinkhof, 2000). Through the combination of neutron reflectometry with other techniques, it can be observed how the method of preparation of these interfaces affects their stability as shown for an α-lactoglobulin-pectin system (Ganzevles, Zinoviadou, van Vliet, Cohen Stuart & Jongh, 2006).

INCOHERENT NEUTRON SCATTERING TECHNIQUES

Inelastic neutron scattering (INS) as well as quasi-elastic neutron scattering (QENS), both making use of the high incoherent cross-section for hydrogen, enable dynamic processes on a molecular scale to be studied such as drug diffusion or internal molecular motion (Bunjes et al., 2007). Dynamical measurements can be conducted on time-of-flight spectrometers or back-scattering spectrometers and the selection is determined by the time-range of interest. Time-of-flight instruments are used to study the fastest processes (e.g. vibrational spectroscopy), followed by backscattering instruments and finally spin-echo for the slowest (e.g. polymer diffusion in bulk or in confined geometry and dynamics in glasses or membranes). However, in practice, it may be necessary to conduct inelastic and quasieastic experiments across a range of instruments to gain a complete picture of the dynamics of a particular system (Teixeira et al., 2008).

Polysaccharide hydrogels are one such example of the application of QENS in which the water diffusion, segmental chain motions and distance fluctuations of the hydrogens in the polysaccharide glycosidic linkages occurring in these matrices may be studied (Cavalieri, Chiessi, Finelli, Natali, Paradossi & Telling, 2006). QENS can also be used to study functionality-related protein dynamics. The role played by fast sub-nanosecond structural fluctuations, which can

be probed by this neutron scattering technique, deserves special attention as the latter are implicated as being essential to activate biological functionality. As H-atoms are quasi-uniformly and abundantly distributed in a protein, neutron experiments provide a valuable experimental approach to study macromolecular dynamics in detail, as hydrogens reflect the motions of the chemical groups to which they are bound. Incoherent neutron scattering has been used to demonstrate that the average rigidity of a protein structure decreases abruptly immediately below the onset of the enzymatic activation (Paciaroni et al., 2005). Naturally, the presence of water on dynamics has a major influence on the incoherent scattering. To distinguish between contributions from hydrogens present in the aqueous phase and those in the biomaterial, the systems can be hydrated with deuterated water. Other food-relevant examples include studies of water dynamics in bread (Sjöström, Kargl, Fernandez-Alonso & Swenson, 2007), influence of moisture content on lysozyme powders (Marconi, Cornicchi, Onori & Paciaroni, 2008), changes in dynamics in fresh and freeze-dried strawberry and red onion (Jansson, Howells & Swenson, 2006) and the molecular motions of glucose (Smith, Price, Chowdhuri, Brady & Saboungi, 2004), alpha-amylase (Fitter, 1999), ascorbic acid (Bellocco et al., 2008) and starch (Di Bari, Cavatorta, Deriu & Albanese, 2001; Di

Bari, Deriu, Albanese & Cavatorta, 2003). The replacement of hydrogen with deuterium, for example in amino acids or lipids, can also be used to selectively highlight dynamics within a system.

Infrared and Raman spectrometers are commonly present in chemistry departments worldwide. These vibrational spectroscopies provide information about atomic displacements in molecular or crystalline materials but require a change in dipole moment for infrared or polarisability for Raman. Thus, some of the vibrational modes may have zero intensity or be forbidden as a result of selection rules. This is not the case for neutron vibrational spectroscopy. The most intense bands in neutron spectroscopy are those involving hydrogen atoms due to hydrogen's uniquely high incoherent scattering cross-section. In addition, in principle, all bands are measurable as a neutron has a finite mass and, when scattered, transfers a finite momentum to the atom undergoing vibration. The energy transfer at which a band appears will be the same as its value if observable in infrared or Raman as it is intrinsic to the molecule and not the technique. It is also relevant to note that inelastic neutron spectrometers have a large vibrational range (16 - 4000 cm^{-1}). As an example, alginates have been studied with neutron spectroscopy to complement IR and Raman studies with the former enabling greater sensitivity to the influence of hydrogen bonding (Ralph, Finch, Sartori

& Parker, 1996-7) in addition to collagen and model polypeptides (Middendorf, Hayward, Parker, Bradshaw & Milleril, 1995). Generally speaking, food-based systems are difficult to study with neutron vibrational spectroscopy as the large number of atoms inevitably results in dense, congested spectra. Nonetheless, the technique has been valuable in studying the dynamics of smaller food-relevant components, for example, nucleic acids. The reader is referred to the recent text by Mitchell, Parker, Ramirez-Cuesta & Tomkinson (2005) that describes the current state of application of this technique to a range of systems including biomaterials.

CONCLUSIONS AND OUTLOOK

Until relatively recently neutron scattering methods were squarely within the domain of physical scientists and perhaps rightly so. However, as with other sciences, major advances may be accomplished by bringing together scientists from complementary disciplines. This is certainly the case when physical and materials scientists interact with food scientists, technologists and nutritionists. There is now a desire for neutron scatterers to engage with the expertise offered by food specialists to help design and improve the quality and nutritional value of food (Gilbert, 2008a; Appelqvist, 2008) and, in an era with ever more advanced neutron scattering

instrumentation, higher flux facilities, improved mathematical models and greater computing power, there has never been a better opportunity to do so.

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CAPTIONS TO FIGURES

Figure 1. The range of elastic neutron scattering techniques, corresponding size range and complementary methods shown in relation to the hierarchical structure of starch.

Figure 2. Neutron scattering length densities for common food-based materials (with permission from Hammouda, 2008; adapted from Jacrot, 1976).

Figure 3. SANS patterns of resistant starch formed from processed high-amylose maize starch in water. Four neutron solvent contrasts have been used: 0% D_2O, (i.e. 100% H_2O), 25% D_2O:75% H_2O, 75% D_2O:25% H_2O and 100% D_2O along with an effective fifth contrast from SAXS. Dot points represent the experimental data that have been simultaneously fitted with a power law and two phase non-particulate model (solid lines).

Figure 4. Core-shell nanostructure in solution showing the possibility of selectively contrast matching either of the phases through changing solvent H_2O/D_2O xomposition.

Table 1 - Neutron and X-ray scattering lengths and cross-sections* for biologically-relevant elements. A scattering length, b_i, is associated with a cross-section such that $\sigma_i = 4\pi b_i^2$. *Natural abundance 95%. coh = coherent; incoh = incoherent.

atom	nucleus	b_{coh} (10^{-12}cm)	σ_{coh} (10^{-24}cm^2)	σ_{incoh} (10^{-24}cm^2)	$b_{x\text{-}ray}$ (10^{-12}cm)
Hydrogen	^{1}H	-0.374	1.76	79.7	0.28
Deuterium	^{2}H	0.667	5.59	2.01	0.28
Carbon	^{12}C	0.665	5.56	0	1.69
Nitrogen	^{14}C	0.940	11.1	0	1.97
Oxygen	^{16}C	0.580	4.23	0	2.25
Phosphorus	^{31}P	0.517	3.31	80	4.22
Sulphur	* ^{32}S	0.285	1.02	0	4.51

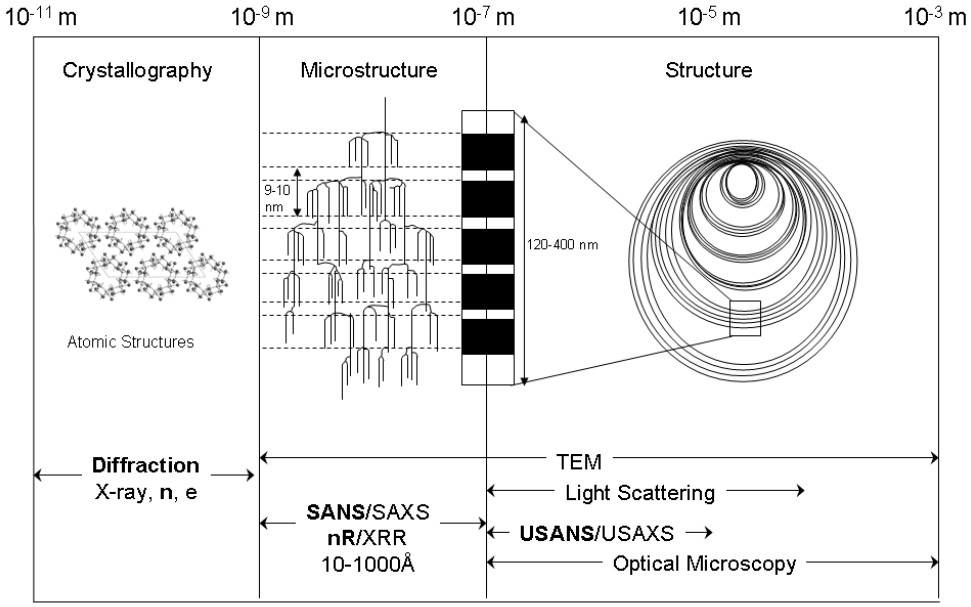
10-11 m
10-9 m
10-7 m
10-5 m
10-3 m
Crystallography
Microstructure
Structure
9-10 nm
120-400 nm
Atomic Structures
Diffraction
X-ray, n, e
TEM
Light Scattering
SANS/SAXS
nR/XRR
10-1000Å
USANS/USAXS
Optical Microscopy

Figure 1

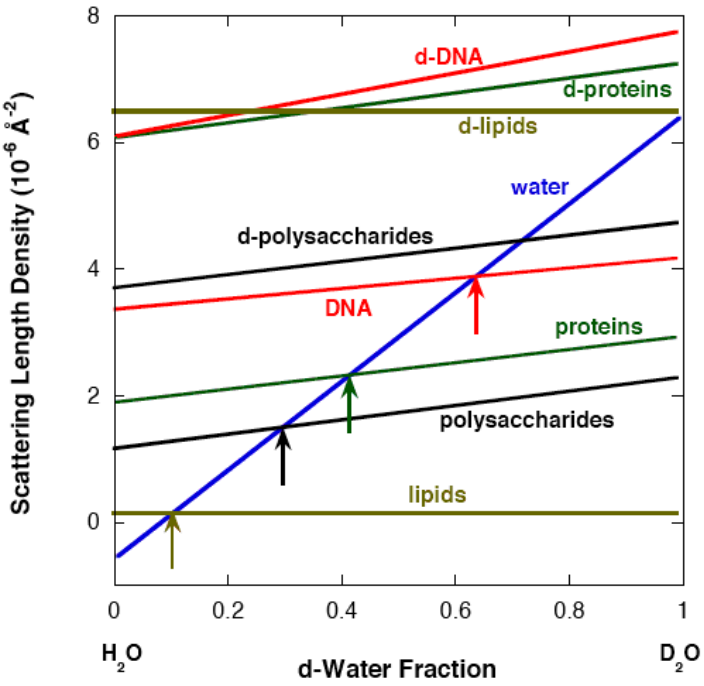
d-DNA
d-proteins
d-lipids
water
d-polysaccharides
DNA
proteins
polysaccharides
lipids
Scattering Length Density (10-6 Å-2)
d-Water Fraction
H2O
D2O
0
2
4
6
8
0
0.2
0.4
0.6
0.8
1

Figure 2

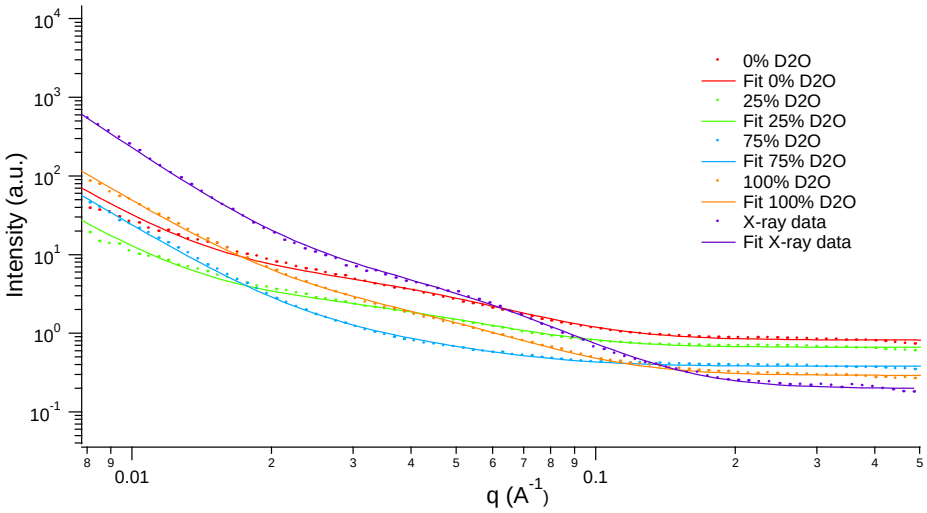
Intensity (a.u.)
q (A-1)
0% D2O
Fit 0% D2O
25% D2O
Fit 25% D2O
75% D2O
Fit 75% D2O
100% D2O
Fit 100% D2O
X-ray data
Fit X-ray data

Figure 3

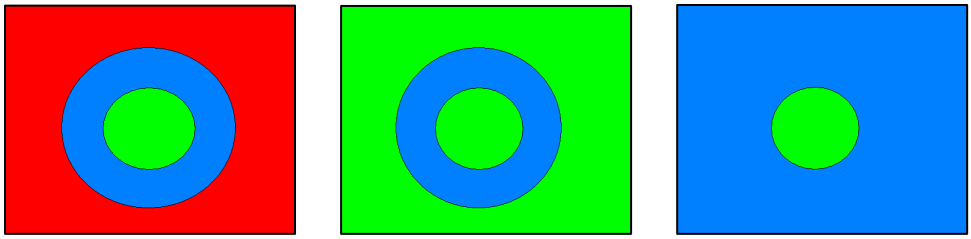

Figure 4