Real Time SAXS/Stress-Strain Studies of Thermoplastic Polyurethane -A fibre diffraction approach to a non-crystalline material

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Elastomeric polyurethanes are synthesised from blocks of flexible, "soft" units linked together with rigid, "hard" blocks. Incompatibility between hard and soft blocks leads to phase separation giving two-phase morphologies with a spatial correlation of the order of 100A that can be monitored by SAXS.

Despite their versatility for a wide range of uses, applications of polyurethanes are still limited by their relatively high mechanical hysteresis compared with other elastomers. This hysteresis and the associated mechanical loss processes can be partly attributed to the breakdown and reformation of the phase structure during mechanical cycling.

This presentation will focus on one aspect of an ongoing programme between Keele and Huntsman Polyurethanes and more recently with Bristol University. It involves measuring the SAXS patterns and stress during tensile deformation in order to follow the changes in morphology and identify effects associated with mechanical loss. It is currently believed that phase separation (on cooling or during solvent evaporation) generally occurs via spinodal decomposition followed by ripening. The exact nature of the final morphology is uncertain and depends on fabrication route and molecular formulation. In attempting to interpret the data we have therefore tried to minimise the number of prior assumptions about the structure. The presentation will describe one approach based on a statistical particulate model which borrows ideas from crystallography.

Axial disposition of myosin heads in contracting muscles

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The development of high brilliance third generation SR sources (e.g. the ESRF) in conjunction with appropriate X-ray cameras and detectors allows the recording of the X-ray diffraction diagrams of muscle tissues with unprecedented resolution and precision. This offers the possibility to study important phenomena such as filament extensibility and symmetry for a variety of short-lived structural states (e.g. Bordas *et al.*, Biophys. J., 77, 3197-3207, 1999). Perhaps still more useful is that the brilliance of third generation SR sources allows the resolving of pronounced interference effects sampling the diffraction diagrams. These effects - due to the bipolar distribution of the motor proteins in the sarcomere - provide phase information and permit a direct visualisation of the disposition of the myosin heads during various forms of contraction (e.g. isometric, unloaded, overloaded, etc., e.g. Juanhuix *et al.*, Biophys. J., 80.)

Co-localisation of type I and type III collagen

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The molecular interactions between type I and type III collagen heterotypic fibrils are essential within some collagenous structures, yet these interactions are not understood to any significant level. The interactions can be modelled and compared to x-ray diffraction information from collagen structures which are rich in both type I and type III collagen (these include skin and the mitral valve complex of

the heart which contain 80% type I and 20% type III collagen). These diffraction studies have shown that the type I : type III complex containing structures have relatively similar x-ray diffraction patterns to that of rat tail tendon which is essentially type I collagen. Yet simulations of type III collagen diffraction patterns show significant differences to that of type I collagen. The intensities of the meridional reflections of the mixed fibrils are attenuated in the higher orders of the diffraction data. The reasons for this attenuation are not yet fully understood, although it could be related to the long-range helical structure of the collagen fibril which appears to be a characteristic of type I, type III heterofibrils. We have built models of heterotypic fibrils in order to simulate their diffraction pattern and to investigate the possible role of type III collagen in the long-range helical structure.

Pore formation studied by neutron reflectometry

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Pneumolysin (PLY), a 53 kDa protein, is a major virulence factor of the human pathogen Streptococcus pneumoniae. It causes damage to lipid bilayer membranes during disease and forms pores within them. Its receptor is cholesterol. We have performed small-angle neutron scattering, specular and off-specular neutron reflectivity studies on the PLY/membrane/cholesterol system to further understand the mechanism of pore formation. How does cholesterol function as a receptor? Does PLY translocate across the entire membrane bilayer? Is leakage of the membrane the result of pore formation by large PLY oligomers or bilayer instability caused by the insertion of individual PLY monomers? The answers to some of these questions will be presented in this talk.

Interactions of cations, water molecules and polysaccharide helices in oriented fibers and correlation with solution properties

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X-ray diffraction patterns from well oriented and polycrystalline specimens recorded on photographic films are our source of experimental data for determining the three-dimensional structures of several industrially useful polysaccharides. In every case, polymer helices, constructed to be consistent with the helical parameters, have been packed in the unit cell appropriately consistent with space group and fiber density constraints and optimized by least squares refinement against the measured intensities. Guest molecules surrounding the helices have been located from electron density maps and the augmented crystal structures further refined to convergence. The emerging structural results in the solid state, in particular the interactions among the helices involving co-solutes and the solvent, help to understand the molecular basis of the observed solution properties of these biopolymers.

In the case of guaran, the galactomannan chain forms a flat, ribbon-like, 2-fold helix stabilized by hydrogen bonds from the galactosyl side-chains to the mannan backbone. Four helices are present in an orthorhombic unit cell. Water molecules promote the formation and subsequent stacking of sheets containing the polymer chains. The naked regions in the galactomannan are the sites of hydration that lead to good solubility and high viscosity in aqueous solution.

In the gellan family of anionic polysaccharides, a half-staggered, parallel, left-handed, 3-fold double helix is always maintained for the gel-forming gellan and the branched polymers welan, S-657 and rhamsan. The interactions involving up to three helices in trigonal unit cells, mediated by cations (e.g. sodium, potassium, calcium) near their carboxylate groups and stabilized by water molecules, correlate with the strong gelling properties of gellan and the thermal stability of viscosity exhibited by the rest.

A recent examination of its sodium and calcium salt forms confirms that iota-carrageenan exists as a half-staggered, parallel, right-handed, double helix. Three helices are present in a trigonal unit cell and their peripheral sulphate groups interact via ions and water molecules in forming the junction zones in carrageenan gels.

Scattering studies of plant cell wall polymers

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The plant cell wall is composed primarily of the polysaccharide cellulose, one of the most ubiquitous biopolymers in the world. Cellulose is the primary building block, and how it packs and responds to external mechanical stresses has important consequences for the viability of the plant. Scattering studies on a variety of cell wall systems will be presented, demonstrating how both long and short range order changes with stress. The consequences of interactions with other biopolymers in the cell wall will also be considered.

Crystallisation from ordered block copolymer melts.

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Structure formation upon crystallization is of fundamental importance to the properties of semi-crystalline solids. We have studied the crystallization of semi-crystalline block copolymers where crystallization occurs from an ordered melt phase. The structure and degree of segregation of the melt phase plays an integral role in determining the structure formed during crystallization. Templating of directional order within the system is preserved in many cases. Results will be presented for a range of systems, morphologies and interfaces.

Structural changes and force generation in muscle cells

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Force generation in muscle originates from the cyclical interaction of myosin cross-bridges with actin filaments, coupled to the hydrolysis of ATP. The shortening of muscle cells is caused by the sliding of interdigitating filaments of actin and myosin, brought about by actomyosin interactions. It is now clear that the elementary movements involve cyclical changes in the structure of the myosin cross-bridges, and in the interactions between myosin and actin. It is these molecular changes which are responsible for the complex mechanical response of muscle fibres to rapidly applied changes in fibre length. Although various crystal forms of the motor domain of the myosin molecule have revealed their atomic structures, there is yet no atomic structure available for the actomyosin complex. Furthermore, protein crystallography does not reveal the dynamics of the structural changes which underlie movement.

Low angle x-ray diffraction of muscle obtained from live or contracting cells provides dynamic, nanometer-scale information which directly relates to the molecular changes responsible for force generation. Although the resolution is only adequate for domain-scale interpretation, and the interpretation remains model dependent, changes in the orientation of muscle cross-bridges on the sub-millisecond time scale can be resolved, as well as changes in the mode of binding of myosin to actin.

The results of recent experiments will be presented in which we show that force generation which accompanies an increase in temperature is caused by stereo-specific binding of myosin cross-bridges to the thin filaments. The shift from non-stereo-specific to stereo-specific binding is also investigated by the use of a myosin-derived, synthetic peptide which

perturbs the equilibrium between actin-attached cross-bridge states.

It will also be shown that cross-bridge binding to actin affects the structure of the thin filaments in a force-independent way.

Neutron reflectivity from model membranes

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Neutron reflectivity has been applied to the study of model biological membranes. Models include flat phospholipid bilayers adsorbed on solid substrates with Langmuir Blodgett/Schaeffer or spin coating techniques. Results of structural determinations from those systems by means of specular neutron reflectivity will be given. Among the examples shown there will be the behaviour at the phase transition (critical swelling) of phospholipid bilayers, the interaction of lipid bilayers with the anti-bacterial peptide alamethicin or with DNA containing cationic liposomes.

Hierarchical structure of collagen and bone studied by x-ray scattering

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Collagen-rich tissues constitute the major structural components conferring mechanical stability to vertebrates. These tissues have exceptional and highly variable properties, a consequence of their hierarchical architecture. At the lowest structural level, collagen consists of triple-helical molecules packed more or less regularly into fibrils.

The structure of the collagen fibrils is not completely solved but most of the information available to date has been obtained from fibre x-ray diffraction measurements with increasing resolution and sophistication. In bone, the fibrils are reinforced by mineral nanoparticles, the size and orientation of which can be studied by small-angle x-ray scattering (SAXS) or fibre diffraction using x-rays or neutrons.

For a better understanding of the mechanical properties, higher levels of hierarchy have to be included. In the case of rat tail tendons, for instance, the strain in the collagen fibrils, measured by in-situ synchrotron x-ray diffraction, is only 1/3 of the total strain of the tendon, or even less. Recently, tendons were studied during tensile stretching using time-resolved fibre diffraction at different strain rates. The aim was to identify contributions from different hierarchical levels to the viscoelastic properties of the tendon.

A different approach was developed for the characterisation of hierarchical tissues, such as bone. In scanning x-ray microdiffraction, the specimen is scanned across a narrow x-ray beam. In this way, structural information is obtained on two scales simultaneously: the one defined by the evaluation of the scattering pattern (that is, the nanometer range) and the one defined by the beam cross-section (corresponding to the micrometer range). With laboratory equipment, bone can be studied by scanning-SAXS using a beam cross-section of 100 micrometer. This lateral resolution of the scanning process can be pushed to a few micometers and even to the sub-micrometer range using synchrotron radiation. By this new approach, structures can be characterised in the range between nanometers and millimeters, contributing to a better understanding of complex biological materials.

Model-independent maximum - entropy inversion of SAXS data

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A model-independent maximum-entropy method is presented which will produce a structural model from 2-dimensional SAXS data of disordered systems using no other prior information. In this respect, it differs from conventional maximum-entropy methods which assume the form of scattering entities a priori. The method is demonstrated using data obtained from perfluorinated ionomer membranes, liquid crystalline copolymers, polyurethanes and other semi-crystalline polymers.

Evidence of spinodal decomposition in semi-crystalline polymers

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Investigations into the early stages of crystallization have been investigated using time-resolved SAXS/WAXS/DSC experimental techniques. The data have been collected for comparative examination from both the ESRF Dubble beam line, France and the SRS 8.2 beam line, UK. These two beam lines enable SAXS/WAXS experiments to be performed on quiescent samples. However, differences in detector instrumentation provides an insight into the developing theory of spinodal decomposition as the mode by which pre-nucleation ordering occurs in semi-crystalline polymer melts. Past arguments have questioned this route to early structure development due to WAXS detector sensitivity limitations compared to SAXS detection.

Several samples of commercial and synthesized polymers such as iPP and PET have been used to provide evidence that SAXS does occur prior to WAXS during isothermal crystallizations. This has led to the theory that primary crystallization can be described as being a phase separation process rather than a nucleation and growth scenario as classical models predict during an initial induction period. Thus, the experimental SAXS data have been seen to follow the kinetics of spinodal decomposition and have been analyzed using the Cahn-Hilliard (CH) linearized growth theory. This describes the time evolution of the scattering intensity to follow an exponential growth from increased amplification of density fluctuations. The fitting of the CH theory to the scattering data gives an extrapolated value for the spinodal temperature. Below this temperature the polymer is said to spontaneously separate into two phases.

Data collected on both beam lines have shown evidence of SAXS before WAXS in isothermal crystallizations of the polymers investigated. The new detector technology with significantly increased sensitivity at the ESRF, has indicated that spinodal decomposition can still be used to describe early structure development during an induction period. However, the increased WAXS detector sensitivity has shown that induction periods are reduced and low isothermal crystallization temperatures have failings when evaluated by the CH theory, compared with pervious data obtained. Nevertheless, the spinodal temperatures obtained for samples still correlate well with past and present data collected on both beam lines.

Astringency - a molecular approach

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Astringency is generally described as a feeling of dryness, puckering or rough mouthfeel and is associated with a number of beverages, including tea, coffee, wine, beer and vegetables and fruits like spinach and dates. All these beverages and foodstuff are similar in that they contain polyphenols.

The sensation of astringency is considered to derive from polyphenols and their ability to bind, and precipitate proteins within the mouth. The major proteins in the mouth are the proline-rich proteins (PRPs), which make up approximately 70% of the protein saliva and contain a high content of proline (25-45 %), glutamine and glycine. These salivary proline rich proteins can be divided in three groups: acidic (30%), basic (23%) and glycosylated (17%) PRPs. The acidic PRPs are believed to bind oral bacteria and to prevent the growth of hydroxyapatite crystals at the surface of the tooth in vivo. It has been proposed that the main task of the glycosylated PRPs is lubrification of the mouth surface and the basic PRPs are considered to bind and precipitate polyphenols.

There are two main structures of polyphenols: Condensed proanthocyanidins and gallic acid derivatives or hydrolysable tannins and minor structures such as phlorotannins and phloroglucinol. The affinity of binding increases with the number of aromatic rings which substantiates the theory that the main binding effect is a hydrophobic stacking of the phenolic ring over a proline, whereas hydrogen

bonds are less important.
After the initial binding three mechanisms have been suggested that lead to precipitation:

*Multiple binding of polyphenols
*Polyphenol-polyphenol stacking interaction
*Multiple binding of the proteins

The formation of aggregates as well as the growing of the complexes is temperature dependent as well as pH dependent.

Swelling of normal and pathological corneal stroma.

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PURPOSE. Corneas swollen in vitro, at physiological pH with a bounding membrane will remain transparent even at pH8 (Huang Y and Meek KM, Biophysics J. 77, 1655-1665,1999) while corneas swollen in vivo become opaque. Our purpose was to compare the swelling of Fuchs' dystrophy and normal corneas to explain this anomalous behaviour.

METHOD.
Experiment 1: Bovine corneas were swollen at pH 8 and pH 5 and final pH3.2 and pH5 using a bounding membrane. Experiment 2 : Normal and Fuchs' dystrophy corneas were either swollen by addition of physiological saline or were equilibrated against different concentration of polyethylene glycol (PEG) in phosphate buffer to a range of final hydrations. The corneas were examined by x-ray diffraction (XRD) and transmission electron microscopy (TEM).

RESULTS. Experiment 1: At pH 8 the corneas at both hydrations appeared clear while at pH 5 the corneas at both hydrations appeared opaque. XRD revealed no correlation between collagen interfibrillar spacing, fibrilar diameter and appearance of tissue. But a correlation was observed between degree of order of the fibril packing and appearance of tissue. A high degree of order was found in the clear corneas while a low degree of order occurred in opaque corneas. Experiment 2: Both normal and Fuchs' dystrophy corneas swelled on addition of saline. All saline-swollen corneas were opaque. Although the final hydrations were different, XRD revealed there was little difference in interfibrillar spacing between Fuchs' dystrophy and normal corneas, but a low degree of fibril packing order was observed. TEM revealed the presence of numerous large intra-lamellar 'lakes'.

CONCLUSION. Corneas swollen in vitro appear opaque because of the formation of collagen-free 'lakes' and an accompanying disordering of the fibril packing. Swelling with a bounding membrane in the region of neutral pH can reduce these effects and allow a more even water distribution and hence a clearer cornea.

Differences in the swelling behaviour of normal and Fuchs' dystrophy corneas may relate to compositional changes in the source of the osmotic force controlling hydration (probably including changes in proteoglycans) manifested here by a different response to equilibration against PEG. The X-ray diffraction and electron microscope results suggest the presence of more 'lake' formation as the Fuchs' cornea swells (compared to the swollen normal cornea) perhaps for the same reasons. The distribution of tissue water, and hence the resulting light scattering in Fuchs' dystrophy corneas, is probably a consequence of these compositional changes, which do not occur when normal corneas are swollen in vitro near neutral pH with a bounding membrane.

Structure, Fluctuations and Transitions of Lipid Model Membranes

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The structural dynamics of lipid bylayers and non-bilayer phases are highly sensitive to their physical and chemical environment. Time-resolved X-ray diffraction covering both the small- and wide-angle region under different solvation conditions, in bulk liposome and solid-supported states, resp., have resulted in a host of novel information. Most strikingly, the liquid-crystalline state of hydrated multilayers shows discrete steps of solvation, which suggests a non-monotonous interaction potential between adjacent bilayers. Such states appear also as

transient intermediates in T-jump relaxation experiments as short-lived intermediates with sub-second life-times. These observations can be interpreted in terms of a foam model of liposomes. Undulation phenomena, as can be quantified by advanced methods of data analysis, play a central role in this model.

Myosin head configuration in relaxed lethocerus fibrillar insect flight muscle from low-angle x-ray diffraction

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Movements in all cells, whether they are muscle cells or not, is mediated by the action of motor proteins, such as myosin heads and kinesin, on filamentous tracks, respectively actin filaments (polymers of actin molecules) and microtubules (polymers of tubulin). In each case, the motor protein is thought to attach to its track, to change its conformation in some way, thus producing movement, and then to detach again ready for another attachment-detachment cycle. In all cases, the cycle is associated with the hydrolysis of ATP. Although a great deal has been learnt in the recent past about the atomic structures of some of these proteins, it is still not clear exactly how movement is produced. Studies of intact muscle have the advantage that some muscles are extremely highly organised and they give rise to detailed X-ray diffraction patterns that, in principle, can be solved to yield details of the molecular organisation in the muscle.

Two particular muscle systems have been discovered that because of their superb degree of order, provide technical advantages in structural studies. In the case of vertebrate muscles, it is the muscles of fish that are particularly useful for this, whereas in the case of the invertebrates it is insect flight muscles that are the most beautifully organised of all.

In the interest of studying the molecular structure and function of muscle, we are trying to determine the 3D structure of actin and myosin filaments and the structural changes underlying contraction and its regulation. Our main approach involves using simulated annealing and local refinement methods developed in a BBSRC-funded study of X-ray diffraction data from fish muscle. Here, these methods are being used with the aim to solve the full unit cell structure in relaxed insect flight muscle and also rigor insect muscle. Actin molecules and myosin heads are particularly well organized in insect flight muscles and give rise to semi-crystalline low-angle X-ray diffraction patterns.

We have already analysed and solved the structure of myosin thick filament in relaxed insect flight muscle. We are now refining this structure and propose to apply these methods to solve the full unit cell, including the actin filaments with troponin and tropomyosin, in both the relaxed and rigor insect flight muscle using new X-ray diffraction data. This will provide direct information on the actin-myosin interface and also on the conformation and the flexibility of the myosin heads in different defined states.

In this meeting, we will present a poster showing our latest results on this project.

Reaction kinetics and morphology development in flexible polyurethane foam

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Flexible polyurethane foams are formed from the simultaneous exothermic reaction between a diisocyanate with a polyether polyol and water. The morphology of these foams is determined by the competition between the polymerisation and the microphase separation of the 'hard' and 'soft' segments. The hard-segments are formed by the water and diisocyanate reaction producing a polyurea, whereas the soft-segments are formed by the polyether polyol chains[1].

Adiabatic temperature rise measurement (ATR) and forced-adiabatic FT-IR spectroscopy (FT-IR) have been employed to simultaneously monitor polymerisation on toluene diisocyanate (TDI) based

flexible polyurethane foam system, varying the content of surfactant and catalyst. The decay of isocyanate is correlated to the polymerisation kinetics[2]. There is a good correlation between the conversion of isocyanate calculated from ATR and FT-IR data. As the catalyst concentration in the formulation is increased, it has been observed that the overall relative rate of reaction increases. However, the overall relative rates of reaction are the same among the foaming systems with different surfactant concentration.

Forced-adiabatic, time-resolved synchrotron small angle X-rays scattering (SAXS) has been employed to investigate the dynamics of microphase separation during the fast bulk copolymerisation[3]. Initially, there is little scattering (homogeneous liquid), and the peak that starts to grow at q*0.06 $Å^{-1}$ after 81 seconds is evidence for the structural development in the TDI sample. The peak position does not change during the whole process. Microphase separation was observed to occur at a critical conversion of isocyanate functional groups and is shown to follow the kinetics associated with spinodal decomposition. The presence of covalent cross-links is observed to delay the microphase separation of the urea hard-segments. Dynamic rheological measurements have been conducted during the bulk copolymerisation via a vane rheometer[4]. The increase of the modulus of the foaming mixture is resulted from the microphase separation of the hard-segment.

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Hanging by a Thread

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Nylons (polyamides, polycaprolactams) are amongst the worlds truly great commodity polymers. First developed by Wallace Carothers and DuPont in the 1930's their use today extends beyond parachutes, ladies hosiery and related textile applications; 35% of world output is now directed into the automobile industry, chiefly for tyre production. The one thing that all these uses have in common is that nylons are excellent polymers to form fibres from.

The physical properties and the microstructure of nylons have been investigated by an array of techniques over the last 25 years, including SAXS/WAXS and SANS. Indeed, these scattering studies are a textbook example of the complementarity between X-ray and neutron methods. Of particular interest has been the effect of hydration on the properties of the fibres and how macroscopic changes can be explained at the microstructural level.

This talk, based on some recent SANS experiments and preliminary analysis, will be concerned with the effect of acid, alkali and surfactants on the microstructure of nylon-6 and the implications for a particular use of this polymer.

Collagen orientation in the human cornea and its implications for eye surgery

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Collagen in the corneal stroma is laid down in the form of lamellae, each containing an array of parallel collagen fibrils. The lamellae are all in the plane of the eye globe, and the direction of collagen in one lamella is very different to that in the adjacent lamellae. Fibrils thus occur in all directions in the

plane of the cornea and give the tissue radial strength. X-ray scattering revealed that, in the human cornea, there are two preferred orientations of the collagen, nasal-temporal and inferior superior. We have been quantifying this orientation across the cornea and into the limbus, where the radius of curvature changes abruptly. The results showed that the orthogonal arrangement of the preferred orientation is maintained across the cornea to within about 1.5 mm of the limbus at which point a continuous change to an annular disposition of collagen occurs. We have examined some of the features of this circum-corneal annulus. By producing a detailed map of preferred collagen orientation in the normal cornea and limbus, we have started to explain the different post-surgical results (particularly with respect to astigmatism) of corneo-limbal incisions made at different points in the tissue and in different directions. In the near future, these techniques could also be applied to understand structural changes that occur in various corneal diseases.

Micro-deformation of polyurethane foams

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A compact device based on a piezoelectric actuator has been developed for the deformation of micron-sized polymer fibres. Specifically designed for the collection of micro-SAXS on the Microfocus Beamline ID13 at the ESRF, the device has a built-in high-resolution force transducer and video imaging system.

Results from a series of time-resolved tensile deformation experiments on single structural units of elastomeric polyurethane foams, the struts, are presented. Measurements of true stress, local strain and correlated SAXS patterns have been possible for the first time and have enabled the calculation of the principal characterising parameters of the polymer comprising the struts together with information on the variation of structure as a function of strain. Pertinent comparisons with similar measurements made on bulk foams are made.

Crystallization from Sheared Melts of Linear and Branched Polyethylenes

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The use of in-situ probes to explore the molecular and morphological changes which accompany polymer processing provides useful insight into the critical stages of such processes. We report on the application of in-situ time-resolving x-ray and neutron scattering techniques to the study of crystallisation of polyethylene and polyethylene blends from sheared melts. The combination of neutron and x-ray scattering provide details on several length scales both during shear flow and subsequent to flow during crystallisation. We have used these techniques to explore the relationships between shear history and the subsequent anisotropic crystal growth. These reveal the massive amplification in anisotropy which occurs on crystallisation and the dependence on a critical shear strain (i.e. shear rate independence) for anisotropic crystal growth. We have used ex-situ transmission electron microscopy to complement these in-situ techniques. We have used the information on the different length scales to construct a qualitative model of the crystallisation process.

Structural mechanisms of self-assembly and polymorphic supercoiling of the bacterial flagellum

Keiichi Namba

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The bacterial flagellum is a helical filament by means of which bacteria swim. Each flagellum is rotated by the flagellar motor at its base and works as a propeller. The filament is not a simply rigid propeller, but is designed to quickly change its helical handedness for alternating the swimming pattern of bacteria between running and tumbling for chemotaxis. Thus, the function of the filament involves a dynamic switching mechanism.

The flagellum is formed by well regulated self-assembly. The assembly starts with a rotor ring formation in the cytoplasmic membrane by FliF. To extracellular side, a short rod assembles on the FliF ring, and continues to the hook and the long helical filament, which grows to as long as 15 mm by self-assembly of about 32,000 flagellin molecules. The component proteins are exported through the central channel to the distal end. Cap proteins are attached to the growing end to promote efficient self-assembly of appropriate proteins. For the filament growth, the cap protein is FliD, also known as Hook Associated Protein 2 (HAP2). The structure of the cap-filament complex and the isolated cap dimer revealed by electron cryomicroscopy and single particle image analysis show a pentameric structure of the cap, composed of a pentagonal lid-like domain and five leg-like domains that extend into the hole at the distal end of the filament. The structures and the interactions between the cap and filament over symmetry mismatch indicate that the cap must rotate by rearrangement of the five leg domains upon every flagellin assembly to prepare always one binding site for the next flagellin assembly. The predicted movements look as if the five-leg table walks up the helical steps at the distal end of the filament tube.

The flagellar filament is a helical assembly of a single protein flagellin, and therefore its helical form is a supercoil. The filament can be described as a tubular structure made of 11 protofilaments. The supercoiling is thought to involve two distinct conformations and packing interactions of the protofilaments. We used X-ray fiber diffraction, electron cryomicroscopy and X-ray crystallography to solve the structures of two types of straight filaments, L-type and R-type, which represent the two distinct subunit packing interactions. The filament structures revealed that the well-conserved terminal regions of flagellin form axially aligned α-helical bundles in a concentric double tubular structure in the filament core. The inner-tube domain folds up into a compact structure only upon assembly and that is how the self-assembly process is regulated. The polymorphic supercoiling is achieved by an axial lengthwise switch with sub-angstrom accuracy in the outer tube domain of the protofilament. We solved the crystal structure at 2.0 Å resolution of a 41-kDa fragment of flagellin, F41, which was prepared by truncating terminal segments. In the crystal, we identified the protofilament structure having the shorter repeat distance corresponding to R-type. By simulated extension of this protofilament model, we identified a possible switch region responsible for the lengthwise mechanical switch within the molecule. We are currently trying to build atomic models of the two straight filaments by using the F41 model and based on the structural data we have collected by X-ray fiber diffraction and electron cryomicroscopy, in order to look into the coupling mechanism of the twist and curvature that defines supercoiled forms of the flagellar filament.

Phase behaviour of block copolymers in solution

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The phase behaviour of triblock copolymers of oxyethylene and oxybutylene are investigated by SAXS, Rheology and Optical Microscopy. As the concentration of polymer is increased the micelles which form pack into various geometric arrays forming gels. Generally they form in the order cubic to hexagonal to lamella with increasing concentration. Rheology and Optical Microscopy

show the existence of such phases. A hexagonal phase has been recognised using optical microscopy for a 45wt% sample of B11E47B11. This phase was confirmed by the rheological data and the SAXS data.

Simultaneous SAXS/WAXS/DSC experiments have been performed on beamline 8.2 of the SRS CCLRC Daresbury Laboratory, and on BM26 DUBBLE at the ESRF. All data were fitted to a Pearson VII function to monitor the d-spacing, peak width, and peak area, and intensity as a function of temperature. A double log plot of the reciprocal intensity versus reciprocal temperature can easily indentify the order-disorder transition temperature, ODT. The peak-to-peak ratio changed in many of the samples during the temperature ramp experiments, indicative of a phase change. It has been shown that the domain spacings get larger on heating the samples, and larger still on reducing the concentration of polymer.

For many of the samples simultaneous SAXS/Couette Cell experiments were performed at beamlines 2.1 and 16.1 of the SRS CCLRC Daresbury Laboratory. An applied constant or oscillatory shear will orient the domains within the sample. This has been done previously by Hamley *et al* (*Macromolecules*, **30**, 5721, (1997)).

For the samples of B25E90B25 at various concentrations this experiment has shown the existence of cubic, hexagonal and lamella phases with changes in temperature as well as concentration. Form Factor analysis has been carried out on some of the data collected. It has been deduced that for 32.5wt% B16E60B16 at low temperature the phase shown is cubic rather than hexagonal which is what the scattering pattern suggests), due to the position of a minimum in the form factor at root 2. From all the rheology, microscopy and scattering data it has been possible to construct phase diagrams for some of the samples studied. Work is now being done to attempt to study the kinetics of these transformations, which will be fitted to an Avrami curve.

Culture of Escherichia coli cells for the investigation of hydration patterns in fully deuterated DNA and synthesis of selectively deuterated DNA analogues for the investigation of base pair geometry in novel conformations of polymeric DNA

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Neutron fibre diffraction, in conjunction with the isotopic replacement of H_2O by D_2O, provides a powerful way of investigating hydration patterns in DNA. The option of selectively deuterating particular residues in the DNA double helix also has huge potential for investigating DNA conformation, although this has not been done in the past because the labelling methods are complex.

Here we describe two kinds of biological study that can be carried out at the ILL: the high-cell-density cultivation of Escherichia coli and the synthesis of selectively deuterated DNA containing a strictly alternating sequence of adenine and thymine residues. In this last case, such sequences have unique biochemical/biological properties and adopt DNA conformations that in many cases can only be observed in the DNA polymers and not in oligonucleotides. The work is now at a stage where a neutron diffraction study of D-DNA containing deuterated adenine residues is foreseen using instrument D19 at the Institut Laue Langevin (ILL, Grenoble).

Determination of the Phalloidin Position in F-actin using Fibre Diffraction Data

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Some small peptides, such as phalloidin etc, bind to F-actin and control the regulation of the cytoskeleton. Detailed knowledge about the control of the cytoskeleton is important for medical science. Lorenz *et al.* refined an F-actin with phalloidin model that was consistent with fibre diffraction patterns from phalloidin-F-actin sols and stereo-chemical constraints by allowing modifications of the crystal structure of actin and the NMR-structure of phalloidin. In this study, we determined the position of phalloidin in a more straightforward way using fibre diffraction patterns and confirmed their model. We developed a simple method of determining a binding position of a small peptide on F-actin at low resolution.

We obtained fibre diffraction patterns from well-oriented F-actin sols with and without phalloidin. We extracted the amplitudes along the layer-lines from the diffraction patterns at a resolution of 8 Å. First, we calculated J_0-differential Patterson map (cylindrical averaged Patterson map) to determine the radial position of the phalloidin. Peak positions in the map approximately correspond to the relative positions of palloidin, showing that phalloidin is located at 8.5-11.0 Å from the helical axis of F-actin. Second, we refined the radial position and determined the orientation of phallodin in a similar way to the crystallographic refinement of heavy atom derivative without knowledge of protein phase information. Although the precise orientation could not be determined with high confidence, two candidates with the fittest orientation were determined. Third, on the basis of the position and orientation of phalloidin, we calculated the phase angles along the layer-lines for F-actin and determined the relative position against the F-actin model, the position along the filament helical axis and the rotation about it, using the phase difference between the calculation and the model. The position along the filament helical axis and the rotation about it were determined with precision of ca 2 Å and 20 degree, respectively. Above all, when the center of one actin subunit in F-actin is put on the x-axis, the position of phalloidin was determined to be located at 8.5-11.0 Å in radial direction, at -1.9-1.2 Å in helical axis direction and at 84-103 degree in the rotation direction. Finally, we refined the orientation and position using the F-actin model and the diffraction data. The final result supports the phalloidin position refined in the Lorenz model.

The three-dimensional structure of type I collagen

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Collagen is the most abundant protein in mammals, where it functions as the main component of the connective tissues which maintain the structural integrity of the organism. We present here for the first time a directly determined three-dimensional image of the arrangement of collagen molecules in the fibrils of tendon in the native state. This is in the form of an electron density map which has been obtained by the multiple isomorphous replacement (MIR) method used in protein X-ray crystallography; it immediately reveals the quasi-hexagonal packing of the collagen molecules and the sites of the cross-linked telopeptides which are central in understanding the function of both normal collagen fibrils and those in connective tissue diseases. It also shows a novel regular arrangement of ordered and disordered domains in the fibril.

The Fab and Fc fragments in the antibody subclass IgA2 exhibit a different arrangement from that in the IgA1 subclass: a study by solution scattering, ultracentrifugation and homology modelling

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Human immunoglobulin A is an abundant antibody that occurs as two major subclasses, IgA1 and IgA2. It mediates immune protection at mucosal surfaces as well as in plasma. Both isotypes contain two four-domain Fab fragments and a four-domain Fc fragment analogous to that in immunoglobulin G. In IgA2 these are linked by a hinge region made up of 8 amino acids from each of the heavy chains, while the length of this hinge in IgA1 is 23 residues. IgA2, like IgA1, also has two 18-residue tailpieces at the C-terminus of each heavy chain in the Fc fragment. X-ray scattering using H_2O buffers and neutron scattering using 100% $2H_2O$ buffers were performed on recombinant monomeric IgA2. The radius of gyration R_G from Guinier analyses is 5.1 nm, which is significantly smaller than that of IgA1 at 6.11-6.20 nm. The cross-sectional radii of gyration R_{XS} were similar for both IgA2 and IgA1. The distance distribution function P(r) for IgA2 showed a single peak and a maximum dimension of 17 nm, while that for IgA1 showed two distinct peaks and a maximum dimension of 21 nm. The sedimentation coefficient of IgA2 was determined to be 6.2 S. These solution data indicate that IgA2 is structurally significantly more compact than IgA1. The homology modelling of the IgA2 structure showed that the intact structure can be readily assembled using known crystal structures for IgG Fab and Fc fragments. We have now initiated an automated curve fit search constrained by these homology models in order to model the experimental IgA2 scattering curves and its sedimentation coefficient. This is based on the use of molecular dynamics to generate random IgA2 hinge structures to which the Fab and Fc fragments could be connected in any orientation. Our results to date show that a large number of stereochemically correct IgA2 models can be generated, a small subset of which is able to satisfy a set of constraints based on the parameters from the scattering and ultracentrifugation data. From these searches, it is concluded that the observed compact IgA2 structure is the consequence of its much shortened hinge peptide length. As this makes IgA2 structurally distinct from IgA1, it is possible that IgA2 may have a distinct immune role from that of IgA1 in plasma and mucosa, and evidence for this will be discussed.

Effect of homopolymer addition to the morphology of a block copolymer in the bulk and in thin films

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Diblock copolymers upon cooling (from the melt) or upon solvent evaporation (from solution) exhibit different microphase-separated morphologies including spheres, hexagonally packed cylinders and lamellar structures. The relative volume fraction of each block along with the Flory-Huggins interaction parameter (c) and the degree of polymerisation dictates the morphology formed, and surface effects are important for determining the ordering process in thin films.

The requirements for the lamellar morphology are that the volume fraction of one block being $0.4 < f < 0.6$, to obtain alternating layers. If asymmetric starting materials are used, it is possible to obtain a lamellar morphology by preparing blends with homopolymer(s). In these blends, the free energy is reduced when the homopolymers segregate to the appropriate domains of the ordered structure reducing the number of unfavorable segmental A/B contacts. The microdomains swell in order to accommodate the homopolymers resulting in transitions from one type of microstructure to another in the process[1].

Solutions of a commercially available diblock copolymer, polystyrene-polybutadiene(PS-b-PB) (SB-83, ~83,00g/mol) with homopolymer(s) were prepared in toluene. The homopolymer(s) was added to the diblock copolymer in order to obtain series of samples with different volume fractions of homopolymer but with lamellar morphology (i.e.,

0.4 < fPS < 0.6). The solutions were spin-cast onto polished silicon wafers, covered with a native oxide layer. The uniform thin films were investigated by X-ray reflectivity to determine their total film thickness and by Neutron reflectivity to determine the lamellar spacing and the distribution of the labeled homo-polystyrene. From the evaporated solutions, bulk samples were prepared and used to determine the *d*-spacing by Small Angle X-ray Scattering, and by Transition Electron Microscopy.

From this work, it was shown that the d-spacing increased upon increase in volume fraction of homopolymer in the blends. From our neutron reflectivity results it was suggested that the distribution of the homopolymer was mixed, with a part of homopolymer located in the middle of the domain and the rest completely solubilised in the domain. The effects of processing rate and thermal history were also studied. The mixed distribution disappeared upon annealling

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Investigation of the phase behaviour of block co-polymer gels containing inorganic salts

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The phase behaviour of aqueous gels of E-B-E (where E = polyethyleneoxide and B = polybutyleneoxide) block co-polymers containing inorganic salts has been investigated. Ternary mixtures of either E17B14E17 or E43B14E43 with water and hexachloroplatinic acid have been analysed using small angle x-ray scattering and rheology. The effect of shear alignment on such mixtures has also been investigated.

Fibre Diffraction Analysis of Fish Muscle Structure

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The crossbridge power stroke on actin appears to involve a change in angle between the actin-attached motor domain and the neck region of the myosin heads. We are using X-ray diffraction methods applied to plaice fin muscle to define these structures in situ, to determine the kinetics of the transitions and to study structural aspects of its regulation by tropomyosin/ troponin. Studies involved are: (i) low-angle X-ray diffraction from resting muscle to define the arrangement and shape of the (M.ADP.Pi) myosin heads on their ordered structure on the thick filament backbone; (ii) low to medium-angle X-ray diffraction with 3D reconstruction of skinned fish muscle labelled with exogenous myosin S1 in the rigor and ADP-bound states; (iii) analysis of actin filament structure using X-ray diffraction data from 'steady state' resting and active muscle; and (iv) fast (1ms) time-resolved low-angle X-ray diffraction from contracting fish muscle during the rising phase of an isometric tetanus to study the kinetics of the crossbridge cycle. The head arrangement in relaxed fish muscle has been defined. Two different myosin head configurations on actin have been identified in active intact muscle. The shift of tropomyosin associated with regulation (steric blocking) has been confirmed and the role of troponin is being determined. Time-resolved X-ray diffraction has started to separate the weak and strong crossbridge states in the contractile cycle and to provide kinetic data between various states. Our best current estimate of the two actin-attached structural states in the fish muscle tetanus is that at the tetanus plateau there are about 46% of the heads in the low-force (mainly weak) state and about 28% of the heads in the high-force (strong) state with 26% detached and resetting.

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Multipole wiggler 6.2 - A world class facility for the study of materials processing

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In collaboration with the University of Aberystwyth, Birkbeck College, the University of Cambridge and the University of Sheffield.

X-ray crystallography achievements have won more Nobel prizes than any other scientific discipline and over the last 50 years during which time X-rays have revolutionised the way in which we visualise and interpret the atomic structure of matter. However, advances in technology mean that scientists using the SRS today want sample cells that mirror real conditions and facilities that are able to follow reactions from one form of a material to another. Pressure, temperature, pH, humidity and the presence of corrosive atmospheres are all part of the current experimental trend.

The centrepiece of the new station, and its most significant development, is the gas multiwire detector combination based on the RAPID technology. Funded by the EPSRC, the curved position sensitive detector for measuring wide-angle scattering has a complex and rapid way of interpolating the diffraction peaks. This enables the detector to obtain a peak resolution of better than 0.06º and data in milliseconds. This timescale and statistical precision will be a much better match for the complementary small-angle scattering and spectroscopy techniques. The new facility will allow the study of reactions, chemical processing and solid state reactions with unprecedented accuracy and millisecond speeds.

The development of MPW 6.2 will keep the SRS at the cutting edge for at least seven years. It is anticipated that the new materials processing facility will be transferred to DIAMOND when the new synchrotron is built.

Microphase Separation of Block Copolymers: Confirmation of the universality of chi.

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It has been known for some time that the molecular architecture at constant chain length and composition will dramatically influence the processes of microphase separation and crystallisation, and consequently the physical properties of block copolymers. The effect of block copolymer architecture on these important processes has been studied in terms of triblock and diblock copolymers of oxyethylene (E) / oxybutylene (B) systems.

Investigation of the microphase separation behaviour of EmBnEm, BnEmBn and EmBn systems was conducted using small angle scattering (SAXS) and rheology. These techniques yielded values for the temperature of the order-disorder transition (Todt) and domain spacings (d) in the ordered phases of the melt. The symmetry of the ordered phase behaviour could be deduced by a combination of 1D and 2D SAXS data analysis and rheology.

Values of the Todt for the BnEmBn triblock copolymers were 100 degrees lower if compared to EmBn diblock copolymers of identical composition and chain length, however they were 30 degrees higher when compared to diblock copolymers with half the triblock length. The d spacing results suggested that the triblock copolymers are 10% more stretched than their corresponding diblock counterpart. The phase diagram was evaluated in terms of chiRv versus the volume fraction of the E or B component, where chi is the flory huggins interaction parameter, and Rv is the length of the copolymer chain defined by the number of segments of given reference volume. The resultant phase diagrams were then compared with theoretical predictions.

X-ray diffraction study of human hair as a model of epithelial tissue

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X-ray diffraction investigations of human hair were carried out using SR of VEPP-3 (Novosibirsk, Russia), the X-ray generator with rotating anode GX-20 (Pushchino, Russia), ID22 and ID27 SAXS/WAXS of ESRF (Grenoble, France). We studied more than five hundred hair samples and carried out more than one thousand different experiments. In the experiments we collected hairs of different age donors from 1 to 92 years with their hair length from 3 to 1000 mm. Scalp-hair samples from donors of different regions with anthropologic ’pressure’ and hair of patients from specialized hospitals were analyzed. The samples of hair were obtained from the following sources: Blokhin Scientific Center of Oncology, Department of Breast Tumor, Moscow; Mammological Institute, Altay Region; the 12 th Municipal Hospital, Department of Gastroenterology, Novosibirsk; Institute of Gynecology and Obstetrics, Novosibirsk B Phthisiology Clinic, Novosibirsk; the public school-pansionat of tundrian nenets, Chelyabinsk 4-th Branch Office of Biophysics Institute. The archaeological samples from Pazyryk mound of mountainous Altay were also investigated (undamaged state of hair samples is due to constancy of cold temperature conditions).

It was shown that the hairs demonstrate a classic X-ray pattern of fiber diffraction. X-ray patterns vary for different people by the presence or absence of diffuse ring of spacing 4.5 nm, the patterns were called ’ring’ and ‘no ring’ respectively. A set of diffraction patterns from ‘no ring’ to ’ring’ can be obtained from the hair of individual donor by scanning along the sample point by point from the root region to the tip. There are some donors, whose

hairs have not got the ‘ring’ X-ray patterns along the hair length which were called ‘ring free’ patterns. We were lucky to discover that a prolonged soaking of hairs in 1M CaCl2 at pH 10-11 can transform the hair sample which initially gave a typical ’ring free’ X-ray pattern in such way that it will be able to produce the ‘ring’ X-ray pattern. It was shown that there is correlation between significant quantities of major and trace elements of endogenous and exogenous origins: Ca-Br, Sr-Br, Ca-Sr.

We propose a two-component structural model of hair tissue, which consists of a flexible component of extracellular matrix (ECM) in series with an inflexible component of keratin intermediate filaments (IF). The weak diffuse arc at spacing 4.5 nm was interpreted as arising from interference between assemblies of flexible ECM units consisting of glycoproteins that can be either fibrillar and ribbon-like or random-coil in morphology and with low electron density.

The positively charged metals can transform the configuration of glycoprotein chains due to electrostatic interaction with multiple anion groups of polysaccharide chains and this results in the increasing of electron density. The structure of keratin intermediate filaments in hair tissue is invariable; the extracellular matrix structure is varied.

Thus, all of epithelial cells display extensive keratin filament frameworks around which the cell shape and polarity are defined. The primary cytoskeletal components in vivo regulate and affect the interaction of epithelial cells and extracellular matrix developing the oriented structure of the whole tissue and at the same time in which flexible extracellular matrix is transformed into fibrillar matrix. Our results propose also the regulatory role of metal content in matrix assembly.

So the hair tissue has to be considered as a structurally continuous organization providing the resistance to mechanical stresses externally applied to the tissue. Mutations that weaken this structural framework and any exogenous factors that change the extracellular matrix increase the risk of cell rupture and cause a variety of human disorders.

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X-ray scattering studies of Archaeological bone; tracking changes in mineral reorganisation

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The archaeological degeneration within the mineral component (calcium hydroxyapatite) of bone structures involves a number of processes probably most important of which are diagenesis, Ostwald ripening and pore formation. The exact nature of these rearrangements is poorly defined although alteration of the crystal habit is known to occur resulting in recrystallisation or accretion of bone mineral. These factors are essential in determining the dynamic changes in bone and archaeological preservation. Small angle x-ray scattering provides information on changes in crystallite organisation size and habit that are ideal for monitoring changes in bone porosity, diagenetic durability and ripening. Recent advances in technology now mean that these local variations can be monitored down to a micron length scale. We have combined measurements from standard x-ray scattering experiments with microfocus x-ray scattering experiments to establish the local changes that can occur to the size and shape of crystallites from a large variery of archaeological bones.

Crystallization of poly(oxyethylene)-b-poly(oxybutylene)/poly(oxybutylene) blends

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Three poly(oxyethylene)-b-poly(oxybutylene) diblock copolymers with different chain length but the same volume fraction of poly(oxyethylene), E76B38, E114B56 and E155B78, were blended with pure poly(oxybutylene) to obtain different morphologies, such as lamellae, gyroid, cylinder, and sphere. The effect of chain length and morphology on the crystallization of poly(oxyethylene) segments in the blends were investigated using SAXS and DSC. It was found that the longer the chain length of the diblock copolymers, the larger was the extent of confinement in crystallization. On the other hand, the morphology also exhibited a marked influence on crystallization. The confinement of the morphology increased in the order: lamellae-cylinder-sphere.