International Journal of Pharmaceutics 417 (2011) 101–111

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Applications of X-ray scattering in pharmaceutical science

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ARTICLE INFO

Article history:
Received 22 December 2010
Received in revised form 11 January 2011
Accepted 17 January 2011
Available online 21 January 2011

Keywords:
X-ray scattering
Structure
Micelle
Liposome
Liquid crystal

ABSTRACT

The use of X-ray scattering techniques in pharmaceutical science is increasing, in part through increased collaborations with the materials science community, and through increased availability of instrumentation, particularly synchrotron sources. The ability to understand not only the biopharmaceutical outcome, but also arguably, more importantly, the structural aspects of drugs and drug delivery systems, is essential to progressing pharmaceutical science; this review serves as an introduction to the major techniques and the wide range of areas in which X-ray scattering may be applied in understanding and controlling structure in pharmaceutical systems.

1. Introduction

Since the early half of the 20th century, X-ray scattering crystallography has been the principal method for determination of atomic level structure in minerals, metals and organic compounds. By mid century, X-ray scattering was used to determine more complex biological macromolecular structures. Consequently, the structures for in excess of 60,000 proteins, nuclei acids, and protein/NA complexes have been resolved using X-ray crystallography. In pharmaceutical systems, X-ray crystallography is now used routinely to determine drug–target protein assemblies to optimise drug design (Lundstrom, 2006). In addition to regular crystallography for drug discovery, X-ray scattering is useful in understanding the structure of pharmaceutically relevant materials such as drug self assembly structures and drug delivery systems. In this article we provide an overview of X-ray scattering, and the wider use of X-ray scattering in pharmaceutical systems.

1.1. Scattering basics

Radiation such as X-rays, neutrons and visible light are forced to deviate from an essentially straight trajectory (i.e. are 'scattered') when they encounter a medium containing one or more localized non-uniformities. The majority of scattering is diffuse in nature, where the angle of scattering is broad and provides limited information on the structure of the scattering element. However, for well ordered structures, the periodic lattice structure scatters radiation in a 'specular' fashion, where a photon from a single incoming direction is scattered in a single outgoing orientation. In this instance, the resulting two-dimensional scattering pattern can then be used to determine the structure of the scattering element.

The scattering behaviour of radiation is dictated by the relative wavelength of the radiation compared to the size of the scattering element. Rayleigh scattering occurs when wavelengths are significantly larger than the dimensions of the scattering element. For example, scattering of visible light from the Sun by the gas molecules in the atmosphere scatter blue light most efficiently. Rayleigh scattering is angular independent and hence limited information can be obtained regarding the structure of the scattering element.

However, electromagnetic radiation or subatomic particles with wavelength comparable to the size of the scattering element are scattered in a more specular fashion. The classes of information of most interest in the pharmaceutical field are particle size and structure on colloidal dimensions (nm–mm), protein structure and solid crystallography (nm–angstrom), hence the radiation wavelength required for analysis must be of comparable size. In addition, the radiation should be non-destructive to the scattering material to allow preservation of the original structure, and be able to penetrate into the materials and provide information about the bulk structure. As such, the commonly used radiation types for structural analysis are X-rays and neutrons (wavelength of the order ~1 Å), whilst visible light scattering (400–700 nm) is generally used for particle size analysis.

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doi:10.1016/j.ijpharm.2011.01.022

1.2. X-ray scattering

X-rays are scattered by the electron clouds of individual atoms in the system. Structural information such as how the atoms pack together, the inter-atomic distance and angle can be obtained by measuring the variation of the intensity of scattering X-rays as a function of the scattering angle θ (discussed further later). The typical X-ray scattering experimental configuration uses a well-collimated X-ray beam of certain wavelength, typically 1.54 or 0.8 Å.

1.3. X-ray sources: benchtop vs. synchrotron

X-rays used routinely in experimental structural studies may be from a benchtop or synchrotron source. Bench-top X-ray scattering instruments generate X-rays using a focussed electron beam accelerated across a high voltage field, which bombards a solid target (Cu or Mo). As electrons collide with atoms in the target and slow down, a continuous spectrum of X-rays are emitted. The X-rays are then monochromatised and collimated. Bench-top X-ray scattering instruments have relatively low flux (typically $\sim 10^8$ photons/s) and hence are generally used for resolving structural information in 'static' equilibrium samples, as acquisition times of minutes to hours are required due to the low flux limiting any useful kinetic information from being obtainable on faster time scales.

Synchrotron radiation is emitted by electrons or positrons travelling at near light speed in a circular storage ring. The loss of momentum of the electrons when forced to bend around the ring is emitted as energy at different wavelengths depending on the type of experiment. Synchrotron X-ray radiation has significantly higher flux (typically $\sim 10^{12}$ photons/s) (Mandelkow and Holmes, 1989) than the bench-top instruments, enabling diffraction patterns with sufficiently useful information to be obtained in milliseconds (Amenitsch et al., 1997). As such, synchrotron-based X-ray sources can be used for high throughput and time-resolved experiments as well as static samples. However, the use of synchrotron X-ray sources is limited to major synchrotron infrastructure and is generally less accessible than benchtop instruments.

The detection systems in both benchtop and synchrotron X-ray scattering facilities are largely similar. Image plates were commonplace until the relatively recent advent of position sensitive gas ionization detectors and more recently CCD and diode array detectors, which coupled with fast computing capabilities allow rapid acquisition times, and fast kinetic studies to be undertaken at synchrotron facilities.

1.4. The link between scattering and structure – Bragg's law and Bragg diffraction

The atomic arrangement within samples with periodic structure, such as a crystalline solid, is described in terms of unit cells. Each repeating unit cell possesses an identical chemical and structural environment. The unit cells are stacked in three-dimensional space describing the bulk arrangement of atoms of the crystal. The three-dimensional structure within each unit cell is described by a set of atomic positions (x_j, y_j and z_j) from the corner (lattice points) of each unit cell, whilst lattice planes describe non-colinear three-dimensional planes of atomic arrangements.

When X-rays or neutrons scatter in a specular fashion the scattered wave fields interfere with each other constructively (overlapping waves produce stronger peaks) or destructively (subtract from one another). For periodic structures such as crystalline arrangements, the waves are scattered from lattice planes separated by the interplanar distance, d. Constructive interference occurs when the scattered waves remain in phase with each other and hence the path length of each wave is equal to an integer multiple of the wavelength. The specular scattering from lattice planes with constructive interference is termed Bragg diffraction and is described by Bragg's law (Bragg, 1913), $2d \sin\theta = n\lambda$, where n is an integer, λ is the wavelength, θ is the scattering angle and d is the interplanar distance. Scattered radiation satisfying the Bragg (constructive) condition will produce very strong intensities known as Bragg peaks in the diffraction pattern, which in turn is used to determine the structural properties of the sample.

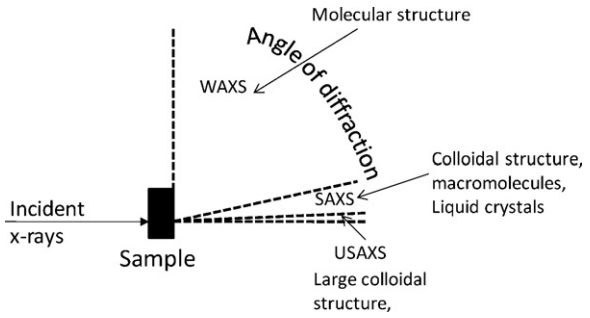

Fig. 1. Schematic showing the broadly classified scattering regimes and the structural levels which they probe.

Based on Bragg's law, with given wavelength, the scattering angle θ is inversely proportional to the interplanar distances. As such, X-ray scattering can give information over a wide range of scattering angles from ultralow angles (0.001–0.3°), small angles (0.1–10°), and wide angle (>10°). These different scattering regimes probe structures at sizes inversely proportional to the angle, e.g. WAXS < 1 nm, SAXS < 1–100 nm, USAXS > 100 nm (Fig. 1).

1.5. Scattering pattern presentation and interpretation

The scattering pattern is often captured as a two-dimensional pattern, and radially integrated to provide the one-dimensional scattering function $I(q)$, where q is the length of the scattering vector, defined by $q = (4\pi/\lambda)\sin\theta/2$, λ being the wavelength and θ the scattering angle (Fig. 2). Alternatively, a one-dimensional line detector may be used rather than a two-dimensional detector, in which case $I(q)$ vs. q profile is obtained directly (after conversion of θ to q). For time-resolved or comparative studies, large numbers of $I(q)$ functions may be compared on the same plot using a 3-D mesh or 2-D contour representation, such as illustrated in Fig. 2.

2. Pharmaceutical applications of wide angle X-ray scattering (WAXS)

WAXS refers to scattering at $\theta > 10°$, which corresponds to spacings at the angstrom or sub-angstrom range. WAXS is therefore suitable for analysing atomic and molecular arrangements, such as crystal structure. Hence WAXS can be applied to pharmaceutical systems to probe crystallinity in drug substances, excipients and drug carriers.

2.1. Protein crystallography

Structure-based drug design (SBDD) is a commonly used method in rational drug design (Anderson, 2003; Williams et al., 2005). SBDD involves identifying the protein and/or enzyme involved in a specific metabolic or cell signal pathway, related to a particular disease state. Knowledge of the three-dimensional geometrical shape or structure of the target protein, allows drug compounds to be designed rationally to selectively interact with the target to bring about the desired effect. Nuclear magnetic resonance (NMR) and X-ray scattering are routinely used to derive the three dimensional structure of proteins and to study drug–protein tar-

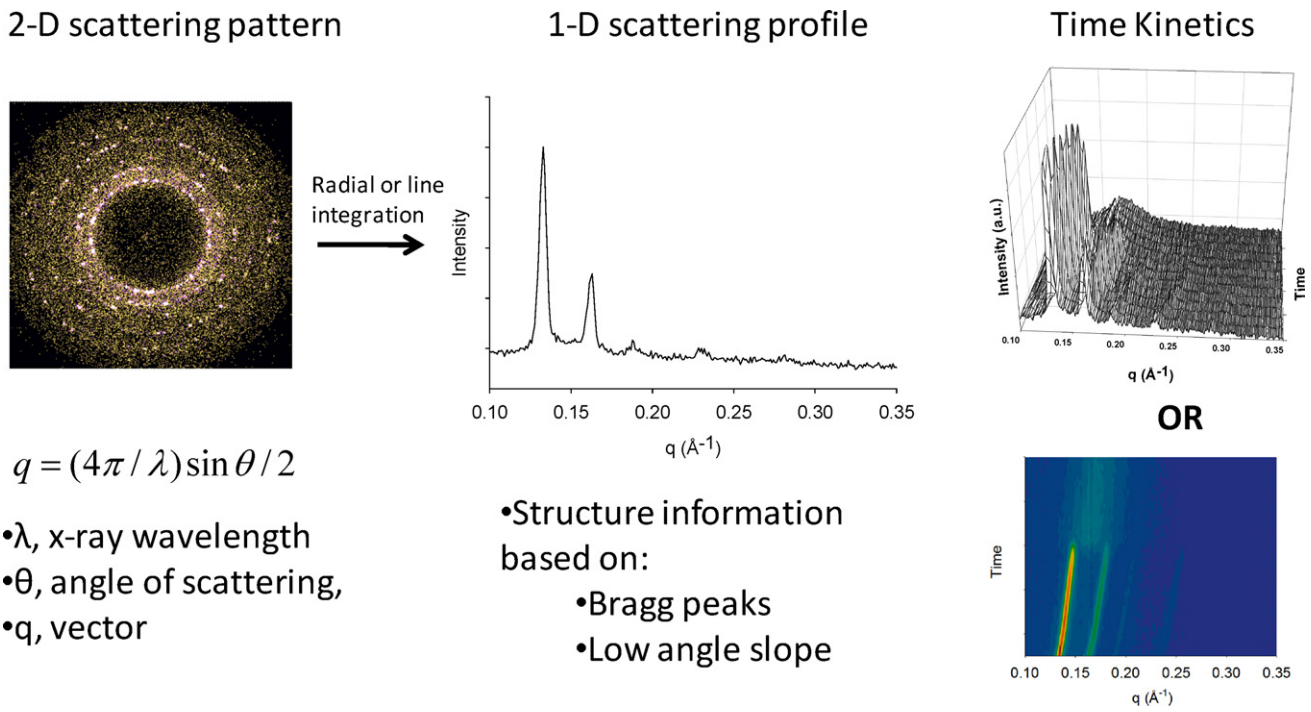

Fig. 2. Schematic of structural characterisation using small angle X-ray scattering (SAXS), in this case the sample is a lyotropic cubic liquid crystal sample. An incident X-ray beam passing through the ordered structure is diffracted at the angle θ to provide the two-dimensional scattering pattern at the detector. Line or radial integration of the pattern results in an intensity vs. scattering vector (q) plot where the Bragg peaks and slope give an indication of structures present. Time-resolved plots are often presented as 3-D mesh or contour plots. The intensity which is represented on the z-axis is represented by the variation in color in the contour plot. In the example, the Bragg peaks from the liquid crystalline structure are lost over time, leading to a broad diffuse hump (in this case representative of inverse micellar structure from melting of the liquid crystalline structure).

get interaction for SBDD optimisation (Deschamps, 2005; Scapin, 2006; Takeuchi and Wagner, 2006).

2.2. Drug crystallization

Polymorphism describes the propensity of a drug substance to exist as two or more crystalline phases that have different molecular arrangements in the crystal lattice. Drug polymorphism can have a significant impact on pharmaceutical properties such as apparent solubility, dissolution rate, and density (Bernstein, 2007; Hilfiker et al., 2006). These properties can directly impact on the quality and performance of drug products, by impacting stability, dissolution, and in some cases bioavailability. X-ray scattering crystallography allows the determination of polymorphic forms, and hence aids in the drug product design and optimisation (Fig. 3).

In addition to the study of polymorphic states, WAXS has been used to study drug solubility. Precipitation of drug from solution occurs via crystallites of drug, which can be detected using WAXS with simultaneous identification of crystalline morphology (Chiou, 1977; Friedrich et al., 2006). Drug precipitation upon administration due to dilution is also an issue for the use of micellar and emulsion systems as it often leads to unpredictable drug bioavailability (Narang et al., 2007). By simulating post-administration conditions *in vitro*, X-ray scattering can be used to detect the existence and morphology of precipitated drug crystals and consequently aid in formulation optimisation (Sassene et al., 2010).

In addition to analysing crystalline formations and polymorphic state, WAXS can be used to quantify the proportions of crystalline forms within a sample. Using reference samples prepared by physical mixture of pure amorphous and crystalline drug at known ratios, it is possible to construct a calibration curve to enable determination of the degree of crystallinity in unknown samples (Kamat et al., 1988; Otsuka et al., 2002).

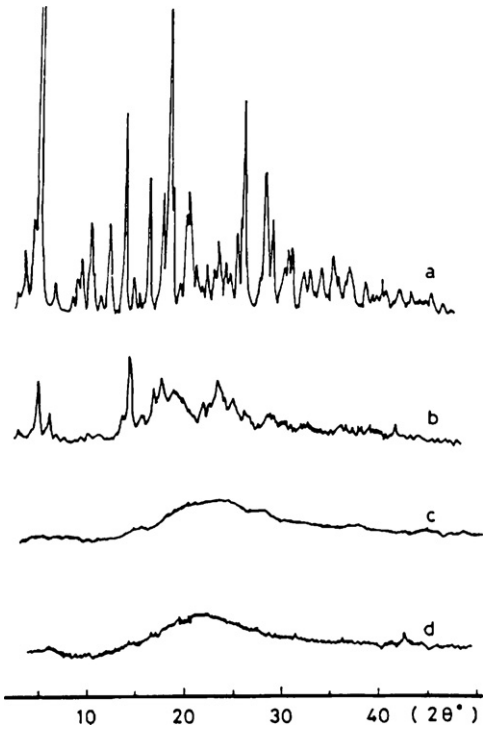

Fig. 3. X-ray diffraction of different forms of solid cephazolin sodium: pentahydrate form (a); dehydrated form (b); amorphous forms from grinding (c) and freeze-drying (d).
Reprinted with permission from Kamat et al. (1988).

2.3. Excipients and drug carriers

In addition to understanding the crystalline structure of drugs themselves, WAXS can be used to characterize and optimize drug carriers and excipients. For example, solid lipid nanoparticles have been investigated as sustained release systems (Schwarz, 1999) for pulmonary (Jaspart et al., 2007), oral (Demirel et al., 2001), IV (Fundarò et al., 2000) and transdermal drug delivery (Müller et al., 2002). Crystallinity of the lipids is reported to have a significant impact on drug loading and controlled release behaviour. Therefore characterization of the physical state of the lipid particles by WAXS and other techniques, such as DSC, is necessary for controlled release optimization. Another example where polymorphism of excipients can influence drug delivery performance is lactose in dry powder inhalers (Traini et al., 2008).

2.4. Other uses of WAXS

In addition to analysis of the crystalline state of drug and excipients, WAXS has been utilized to determine crystallographic transformations taking place on the surfaces of tablets due to differences in compression pressure (Koivisto et al., 2006). WAXS has also been proposed as a quick, non-invasive technique for detecting counterfeit drug products by comparing the diffraction patterns from genuine tablets and the samples in question (Maurin et al., 2007).

3. Pharmaceutical applications of small angle X-ray scattering (SAXS)

Small angle X-ray scattering, as the name implies, is used to detect scattering at angles $\theta < 10^{\circ}$, which corresponds to interplanar distances with nanometre dimensions. This size range contains information about the shape, size and internal structure of macromolecules and longer range structures, such as those found in lyotropic liquid crystals and mesoporous materials.

3.1. Size, shape and interfacial properties

In the introduction, it was mentioned that scattering occurs when radiation encounters a medium containing localized non-uniformities. In the case of two-phase samples such as particles in liquid suspension, the differences in electron density ρ, at the interface between particles and the continuous medium produce scattering at higher angles in the SAXS regime. The scattering by the interface provides information such as surface area, smoothness and thickness based on Porod's (1951, 1952) law and its modifications/deviations (Hummel et al., 1988; Kim, 2004; Ruland, 1971).

Conversely at the lower angle end of the SAXS range, the so-called Guinier region can provide insight into the radius of gyration of any distinct structures (Guinier, 1959). When sufficiently dilute, so that aggregation is minimized, the scattering in this region follows the Guinier approximation. The size and shape of the macromolecules in question can then be determined by modelling of the scattering at low angles (Putnam et al., 2007; Saraf, 1989).

The freely available book 'small angle X-ray scattering' edited by Glatter and Kratky (1982) extensively reviews and summarises the background and techniques in the use of SAXS for determining the size, shape, interfacial properties and surface structures of various systems. It is highly recommended that those interested in the use of SAXS download this book from http://physchem.kfunigraz.ac.at/sm/.

3.1.1. Proteins in solution

Crystallography provides precision high-resolution protein structures, in the crystalline state, however the relationship between the crystalline structure and conformational state under physiological conditions is not readily determined. Protein crystallization required for protein crystallography often requires high concentrations of organic polymers, salt, and additives. Such conditions are very different from physiological systems and, as such, can alter protein–drug interactions. Although SAXS has lower resolution (>1 nm) and hence cannot provide information on structure at the atomic level, it can provide information on gross structural features such as shape, quaternary and tertiary structures under physiological conditions, and insights into protein function (Fig. 4). SAXS has been used to characterize size and shape of biological macromolecules such as RNA (Rambo and Tainer, 2010), proteins (Chacón et al., 2000; Hura et al., 2009), and protein complexes (Sardet et al., 1976) in biologically relevant solutions, and for the study of the effects of solution conditions on conformation (Ianeselli et al., 2010; Zhang et al., 2006). Furthermore, synchrotron SAXS can also provide time-resolved structural information, for example during protein folding or nucleotide hydrolysis (Davies et al., 2005; Kataoka et al., 1997; Zhu et al., 2004). The use and progress of SAXS for biological macromolecules such as RNA and proteins in solution has been comprehensively reviewed (Lipfert and Doniach, 2007; Svergun and Koch, 2003).

3.2. Self assembled systems

3.2.1. Micelles

Micellar systems are a common drug delivery vector for poorly water soluble drugs, enabling sufficient dose to be solubilized in a practical volume of aqueous medium (Kataoka et al., 2001; Torchilin, 2001). They are also an important intermediate structure in the absorption of drugs and poorly soluble nutrients from the GI tract (Carey and Small, 1970). Hence the structure of micelles, and the impact of drug solubilization on structure is an important aspect of pharmaceutical science. SAXS can provide information such as size and shape of micelles as well as micelle aggregation number, radius of gyration and characteristic inter-headgroup spacing across the micelle core (Lipfert et al., 2007). Synchrotron SAXS has been used to observe the formation and transformation of micelles in real time (Hirai et al., 1995a, 1996; Liu et al., 1999; Lund et al., 2009; Schmolzer et al., 2002; Weiss et al., 2005).

In the pharmaceutical field, SAXS has been used to determine the effects of drug (Mackeben and Müller-Goymann, 2000) and enzyme loading (Papadimitriou et al., 1994) on the structure of micelles. The effects of molecular structure of monomeric amphiphiles on micellar formation and structure have also been investigated (Dupuy et al., 1997; He et al., 2002; Zhang et al., 1999), aiding in selection of amphiphiles for formulation optimisation. Such studies may also provide insight into micelle interactions with other endogenous amphiphiles such as bile salts and help to predict the fate of micelles after oral ingestion. SAXS studies on the effects of counterions (Joshi et al., 2007) and non-aqueous solvents (Aizawa, 2010) on micelle structures also provide useful information for optimisation of micelle-based drug formulations.

3.2.2. Liquid crystalline drugs and carriers

Molecules often align in the liquid crystalline state, providing order in one or more dimensions, whilst maintaining liquid-like properties in other dimensions (Müller-Goymann, 2002). There are two principal types of liquid crystals: thermotropic liquid crystals (TLCs) and lyotropic liquid crystals (LLCs). TLCs can be formed by heating a crystalline solid or by cooling an isotropic melt, whilst LLCs can be formed by certain amphiphilic molecules in the presence of solvents, usually water.

Given the longer range of periodicity in liquid crystals compared to solid crystals, particularly in the case of lyotropic liquid crystals, SAXS and/or WAXS are often used to determine the internal nanos-

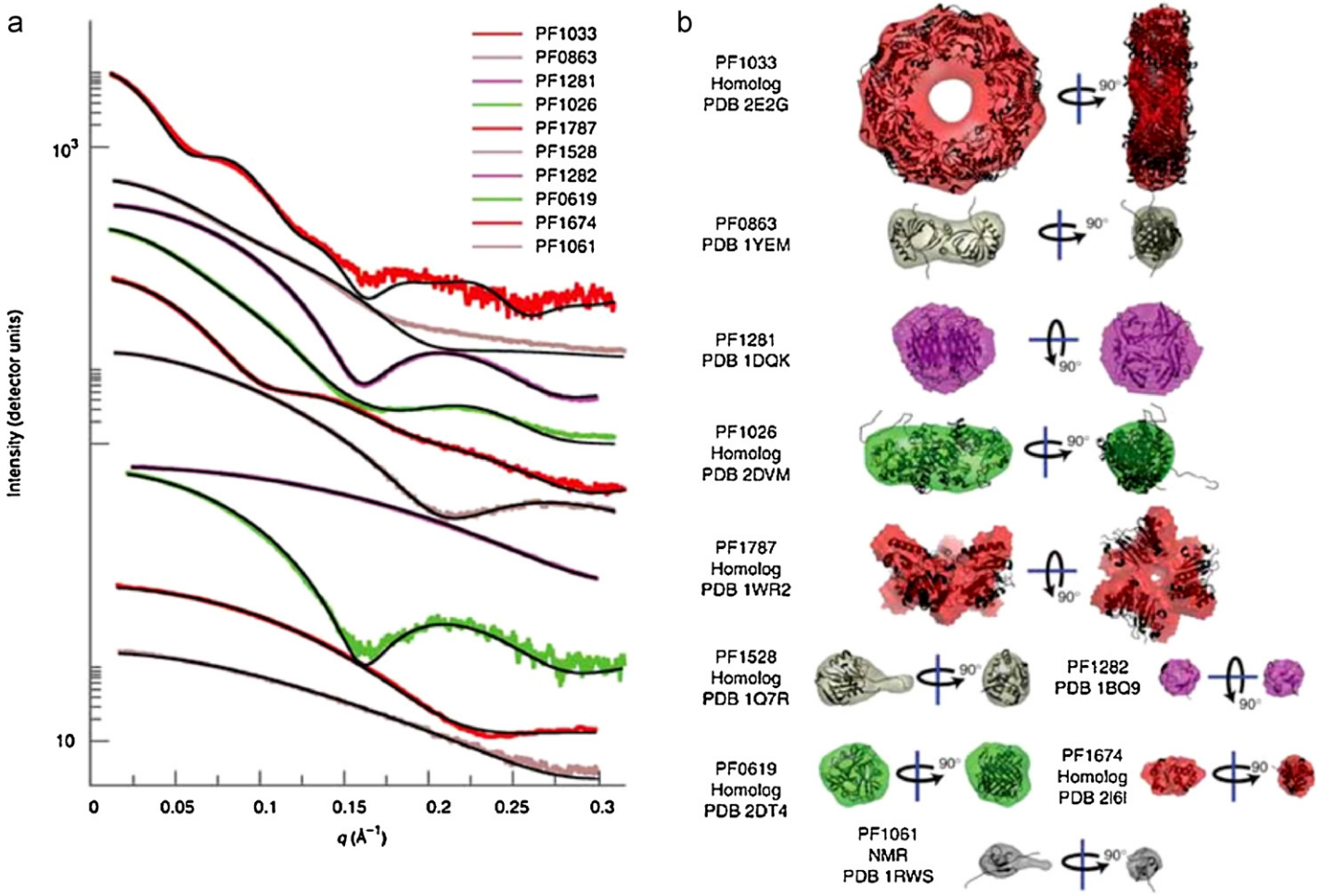

Fig. 4. (a) Scattering curves *calculated* (black) from protein structural homologs or existing structures compared to the *experimental* scattering data (colors) and (b) the envelope determinations based on scattering (colored as in a) were overlaid with the existing structures (ribbons) indicating SAXS provided accurate protein structural information. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
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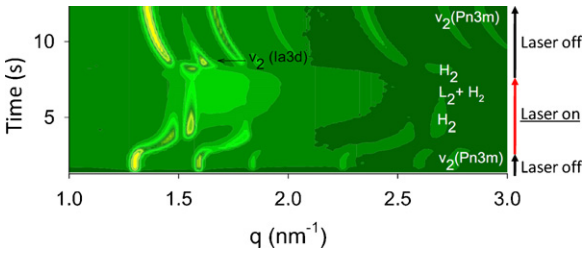

Fig. 5. Time resolved synchrotron small angle X-ray diffraction profiles for the phytantriol + water liquid crystalline system with imbedded gold nanorods (GNR), in response to laser activation. Increased color intensity towards bright yellow indicates increased intensity of signal at that *q*-value. Annotated phase structures (inverse cubic, v_2(Pn3m); inverse hexagonal, H_2; inverse micellar, L_2) determined from indexing peaks in intensity vs. *q* profiles.
Adapted with permission from Fong et al. (2010).

tructure of LCs. In addition to structure identification, synchrotron SAXS has been used to investigate the formation and transformation of LLCs in real-time to provide further understanding and control over their self-assembly behaviour (Dong et al., 2010; Fong et al., 2009, 2010; Squires et al., 2000; Yaghmur et al., 2008a,b).

Liquid crystalline structure may be important in terms of both the state of the drug, or the use of liquid crystalline materials as a drug delivery matrix. Manipulation of drug structure to form liquid crystals instead of solid crystals, such as thermotropic liquid crystals, has been investigated as a possible solution to enhance the apparent solubility and dissolution rate of drugs (Patterson et al., 2002; Rades and Müller-Goymann, 1994). Case studies of thermotropic mesomorphous drugs and pharmaceutically relevant molecules have been reviewed by Bunjes and Rades (2005). Certain drugs with amphiphilic properties can also self-assemble in the presence of water, to form LLCs or micellar structures (Gutiérrez-Pichel et al., 2003; Mukerjee, 1974). Examples of surface active, self-assembling drugs include antivirals (Rodriguez-Spong et al., 2008), phenothiazines (Attwood et al., 1974), non-steroidal anti-inflammatory drugs (Fini et al., 1995), among others (Schreier et al., 2000). The self-assembly of amphiphilic drugs may also affect their properties such as chemical stability (Kurz, 1962; Wallace et al., 2010). As such, X-ray scattering can provide useful insight into assembly behaviour of these drugs, and aid in formulation optimisation.

Lipid-based lyotropic liquid crystal systems comprise discrete lipophilic and hydrophilic regions in a continuous or discontinuous matrix (Yaghmur et al., 2005). They have been considered as promising drug delivery systems for some time (Drummond and Fong, 1999; Engstroem, 1990; Ericsson et al., 1991; Shah et al., 2001). Studies have demonstrated that the nanostructure of the lyotropic liquid crystal systems can have a significant bearing on the controlled release characteristics of the matrix (Fong et al., 2009; Lee et al., 2008) which in practical terms can be controlled by temperature, additives or for some systems, pH (Borne et al., 2001; Caboi et al., 2001; Chang and Bodmeier, 1997; Clogston et al., 2000; Dong et al., 2006; Engstroem and Engstrom, 1992; Nakano et al., 2002). The thermodynamically stable phase structures formed by lyotropic liquid crystal systems has also stimulated recent advances towards their use as stimuli responsive drug delivery systems which may release drugs on demand (Fong et al., 2009, 2010; Yaghmur et al., 2008b). Fig. 5 shows a contour plot of scattering obtained for a light sensitive liquid crystalline matrix using synchrotron SAXS with millisecond resolution, showing the reversibility of the phase structure to return to the V_2 phase after activation with a laser (Fong et al., 2010). Because the nanostructure is the key to performance of these materials, SAXS is an essential tool to understand the behaviour of the nanomaterials at equilibrium and under influence of stimuli.

3.2.3. Liposomes and bilayers

Liposomes are artificially prepared vesicles made of lipid bilayers. Liposomes were first proposed as drug delivery vehicles by Gregoriadis (1973). Since then, liposomes have been extensively used for various delivery applications, including commercially available pharmaceutical products since the early 90 s (Davidson et al., 1991; Guaglianone et al., 1994), and since have been developed for the encapsulation of chemotherapeutic agents (Niu et al., 2010; Pili et al., 2010; Van Bommel and Crommelin, 1984), anti-infectives: amphotericin B (van Etten et al., 1995), vaccines (Gregoriadis et al., 1999), hormones (Shahiwala and Misra, 2004), immuno-modulators (Guo et al., 2001), analgesics (Hung et al., 1995), etc. (Zhang et al., 2007). Liposomes have also been proposed as an encapsulating agent for carboranes, in boron neutron capture therapy (Ristori et al., 2005; Salvati et al., 2007; Soloway et al., 1998).

The liposomal encapsulation and release of drug molecules is governed by drug lipophilicity, lipid composition and structural characteristics such as particle size, bilayer number, thickness and repeat distances (Betageri and Parsons, 1992; Kulkarni et al., 1995). SAXS can be used to study liposome bilayer thickness, bilayer number for multi-lamellar liposomes, and particle size (Bouwstra et al., 1993; Glatter and Kratky, 1982). Synchrotron SAXS has also been used to study the dynamics of the self-assembly of liposomes from micelles (such as the example in Fig. 6) (Weiss et al., 2005, 2008), and their interactions with other molecules (López et al., 2002; Schmolzer et al., 2002). SAXS has also been used to investigate the effects of drug loading on liposome structure (Ristori et al., 2005; Salvati et al., 2007; Schütze and Müller-Goymann, 1998; Wörle et al., 2006).

The surface of liposomes can be conjugated with specific ligands such as proteins and antibodies to enhance targeting or modulate desired immune response (Heath et al., 1981; Martin et al., 1981; Oja et al., 2000). It is possible to distinguish between proteins encapsulated inside the liposome from those at the surface using SAXS (Bouwstra et al., 1993; Skalko et al., 1998).

Liposomes can also be modified to enable drug release to be triggered at the desired site of action, to maximise drug efficiency and minimise toxicity. Potential stimuli that can be employed for triggered drug release include electromagnetic-fields (Viroonchatapan et al., 1997; Zhu et al., 2009), light (Paasonen et al., 2007, 2010; Shum et al., 2001; Yavlovich et al., 2009), ultrasound (Schroeder et al., 2009), pH (Kim et al., 2009; Simões et al., 2004), and temperature (Lindner et al., 2004; Paasonen et al., 2007). The mechanisms for triggered drug release rely on structural changes of the encapsulating liposome. Synchrotron SAXS is a particularly powerful technique in studying such systems, as changes in liposome structure in response to stimuli can be observed in real-time and correlated with release of active substances (Paasonen et al., 2010; Yaghmur et al., 2010). Such a correlation is illustrated below in Fig. 7, where transformation from liposome to an inverted hexagonal phase stimulates release of an encapsulated agent.

3.2.4. Microemulsions

Microemulsions are clear, stable, isotropic mixtures of oil, water and surfactant, frequently in combination with a cosurfactant (Lawrence and Rees, 2000). Microemulsions have received great interest as drug and enzyme delivery systems (Ghosh and Murthy, 2006; Lawrence and Rees, 2000), due to their capacity to incorporate a wide range of drug molecules, they can often be filter-sterilized due to small particle size compared to conventional emulsions, and possess high colloidal stability.

USAXS and SAXS have been employed to investigate the structure of microemulsion systems (Barnes et al., 1988; de Castro Dantas et al., 2009; Glatter et al., 2001; Hilfiker et al., 1990; Hummel et al., 1988; Nakamura et al., 1999; North et al., 1990, 1986;

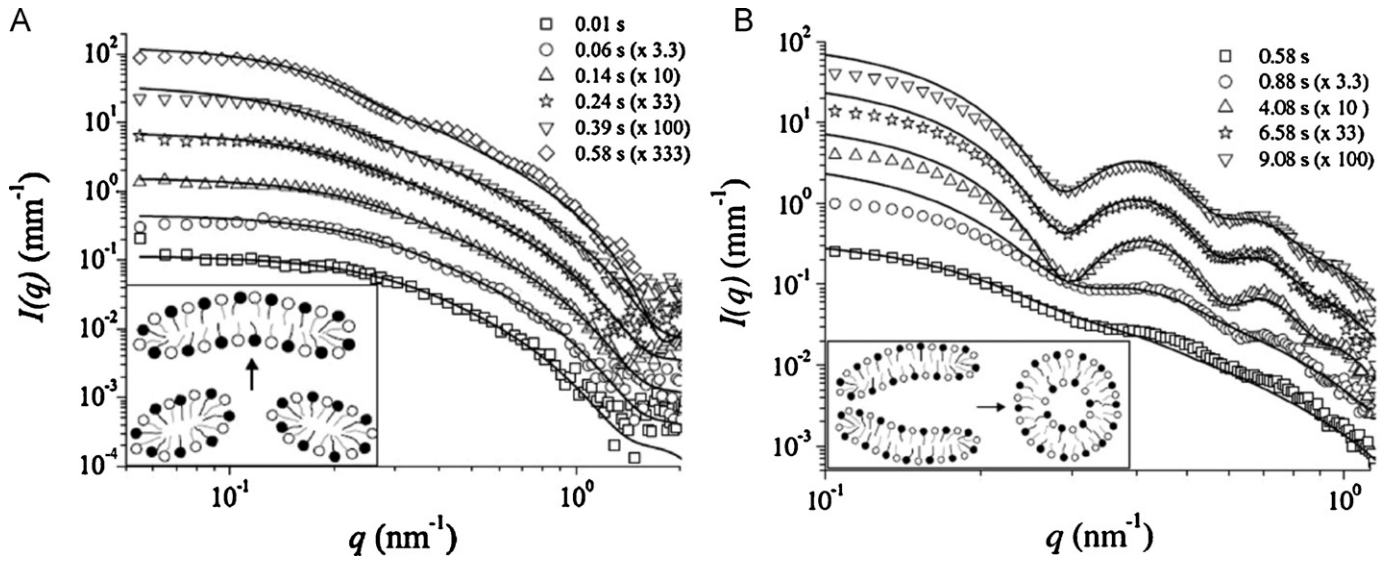

Fig. 6. The evolution of $I(q)$ indicating the growth of disk-like micelles from normal micelles (a) and from disk-like micelles to unilamellar vesicles (b). Adapted with permission from Weiss et al. (2005).

Regev et al., 1996; Shimobouji et al., 1989). Synchrotron SAXS has been used to study the interaction of microemulsions with proteins (Hirai et al., 2002, 1995b) and polymers (Hilfiker, 1991). Microemulsions have been used as transdermal drug delivery systems with potential to enhance drug penetration. Studies have indicated that the internal structure of microemulsions can influence cutaneous delivery from these vehicles (Kreilgaard, 2002). However, the relationship between microemulsion structure and delivery efficiency has not been fully established.

Microemulsions are also employed as a template for the production of other drug delivery systems. For example, poly(alkylcyanoacrylate) (PACA) nanoparticles have gained extensive interest as bioactive carriers, including proteins (Watnasirichaikul et al., 2000). The nanoparticles are often produced via interfacial polymerisation of w/o microemulsions (Gasco and Trotta, 1986). The effect of microemulsion structure on production of PACA nanoparticles was investigated and a surprising dependence of the resulting nanoparticle product on microemulsion structure was found (Krauel et al., 2005), highlighting the potential additional benefit of using SAXS to correlate structure with performance in microemulsion templating systems.

3.2.5. Mesoporous materials

Ordered mesoporous silica materials due to their highly ordered structure, larger pore size and well designed surface properties have received attention as potential pharmaceutical delivery agents (Vallet-Regí et al., 2007). The use of mesoporous materials for drug delivery has been comprehensively reviewed (Vallet-Regí et al., 2007; Wang, 2009). SAXS has been used extensively to determine structural properties such as pore-size, open or closed pore state, and interfacial characteristics (Fall et al., 2010; Li et al., 2001).

The pharmaceutical performance of mesoporous materials, such as drug loading (Song et al., 2005; Zhu et al., 2005) and release kinetics (Horcajada et al., 2004; Izquierdo-Barba et al., 2005) is influenced by pore structure (cubic structures (Andersson et al., 2004; Izquierdo-Barba et al., 2005) and hexagonal structures (Andersson et al., 2004; Doadrio et al., 2006)), pore size (2–50 nm) (Horcajada et al., 2004) and surface properties such as functionalisation (Hoffmann et al., 2006).

Similar to liposome and liquid crystalline systems, mesoporous materials can be tailored for triggered release using a range of stimuli (Yang et al., 2005; Chang et al., 2005; Aznar et al., 2009; Lin et al., 2001; Arruebo et al., 2006; Descalzo et al., 2006). Triggered release often involves changes to the mesoporous structure, such as the opening or closing of the pores. Such changes may be observed, in real-time via synchrotron-based SAXS and correlated to drug release as for lipid based systems.

Mesoporous materials are often prepared from supramolecular assemblies of surfactants which template the inorganic component (usually silica) during synthesis (Vallet-Regí et al., 2007). The

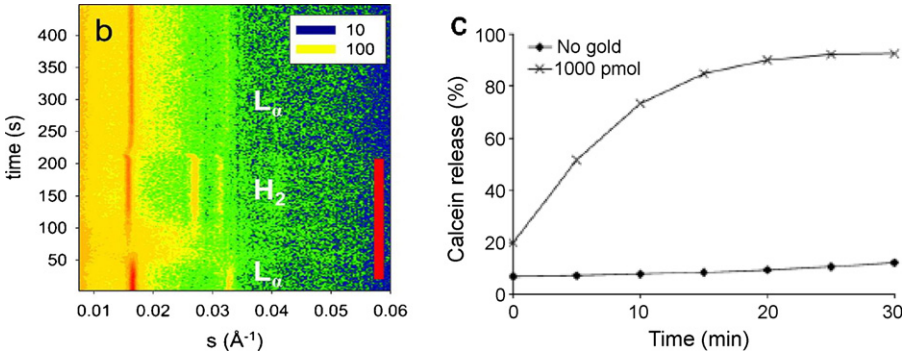

Fig. 7. Left panel (marked b) illustrates SAXS profiles of liposomes undergoing L_α to H_2 phase transition under the influence of UV light (the phase transition was caused by UV-induced heating of embedded gold nanoparticles), modified from Yaghmur et al. (2010). Right hand panel shows release of calcein in the absence (circles) and presence (crosses) of nanoparticles in liposomes as a function of irradiation time, modified from Paasonen et al. (2007).

structure of the self-assembled template therefore dictates the resulting structure properties of the mesoporous materials. SAXS can be used to study the structural relationship between the template and subsequent mesoporous material formed to optimise performance (Wei et al., in press). Synchrotron SAXS has already been used to observe the formation of mesoporous systems *in situ* (Flodstrom et al., 2004; Morell et al., 2004; O'Callaghan et al., 2010).

4. Conclusion

Scattering techniques provide the pharmaceutical researcher to study structure in self assembled drugs and in structural carrier systems, across a wide range of materials. Whilst they are already in wide use in materials science, the application of scattering techniques beyond structure based drug design and development is only now gaining momentum due to an increased number of instruments, particularly synchrotron sources, available to researchers. The links between composition and structure, and structure and performance are too often bypassed in favour of the simpler composition–performance correlation, which in turn does not provide the necessary understanding to enable optimization of drug assembly and materials for drug delivery. This article has hopefully provided insight into how scattering approaches may be utilised, and stimulate researchers to seek a deeper level of understanding about the structural aspects of drugs and drug delivery systems.

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