

PROGRESS REPORT FOR AINGRA09120

PROJECT TITLE	Design of light-responsive liquid crystalline materials for biomedical applications using SAXS	
INVESTIGATOR(S)	Institution and Department	
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ANSTO Investigators	Tracey Hanley	
Specialist Committee	M	

SCIENTIFIC OBJECTIVES

This application aims to demonstrate the utility of lipid-based liquid crystalline materials as reversible light responsive drug delivery systems. Drug release from nanostructured systems has been shown to depend critically on liquid crystal nanostructure and can be switch 'on and off' through induced changes between mesophase structures. SAXS will be used to monitor changes in liquid crystal structure on exposure to light of particular wavelengths. The sensitivity to light will be imparted by inclusion of photoisomerising molecules which, upon changes in isomer state will induce changes in lipid packing, and hence changes in nanostructure of the materials.

PROGRESS REPORT and RESEARCH OUTCOMES

Two approaches have been taken in order to create light responsive liquid crystal systems; firstly, the incorporation of photothermal materials (gold nanorods (GNRs) to trigger phase transitions through a change in temperature, and secondly, the incorporation of photochromic materials (isomerizing dyes) into lyotropic liquid crystals to initiate phase changes as a result of a steric disturbance of the intricate LC nanostructure. A few lipids have been used in this investigation, including glycerol monooleate (GMO), phytantriol (PHYT), monoelaidin (ME) and Selachyl alcohol (SA), however the most success was encountered with PHYT and GMO systems; these will be the main systems discussed in the results.

The grant has been applied in two sets of experiments. Firstly it was used to obtain the equilibrium phase data on heating phytantriol cubic phase containing gold nanorods in Figure 1. The gold nanorods caused a slight reduction in the temperature of the cubic to H₂ phase transition, but has no effect on the lattice parameter of the nanostructure at fixed temperature. This data was extremely important in converting synchrotron laser irradiation data from lattice parameter vs. time into lattice parameter vs. temperature. The data in Figure 3 is the result of this transformation and has given us great insight into the potential utility of plasmonic nanorods in providing light sensitivity to the materials. In summary, the SAXS time at ANSTO contributed directly to our major findings that inducing plasmon resonances of GNR embedded in liquid crystalline matrices produces localised plasmonic heating of the hybrid matrix, enabling fine control over matrix nanostructure. The phase transition resulting from photothermal heating was fully reversible, and specific to the nanorods/laser wavelength combination. Localized plasmonic heating of the LC did not compromise the integrity of the lipid molecules in any of the mesophases. These findings represent a major significant advance towards viable effective light activated drug delivery systems.

The second set of experiments involved trying to detect kinetic changes in liquid crystalline systems on irradiation of photochromic dyes including azobenzene, spiropyrans, and cinnamic acids incorporated into LC systems. Samples were prepared by mixing known amounts of additive in lipid; water was then added in excess and samples were allowed to equilibrate for at least 12 hours. Dynamic SAXS studies were performed on the Hecus instrument at ANSTO. White light and UV illuminations were conducted using an Abet Technologies Arc lamp fitted with a water filter and either a 420 nm Schott glass cut-off filter for white light or an Edmund Optics U-340 band pass filter for UV. Samples were held at temperatures close to their v₂ to H₂ transition temperatures (~45°C) to maximise the possibility of inducing phase changes. When at equilibrium, samples were illuminated with either UV or white radiation and then switched to the other after 20 min. 100 sec frames were taken of the systems during these studies. The lipids and additives investigated are tabulated below:

Table 1 – Lipids and additives used in photochromic systems

Lipids	Photochromic Additives
Phytantriol (PHYT) Glyceryl monooleate (GMO)	Azobenzene (AZB) 4-Decyloxy azobenzene (4-AZB) 1',3'-Dihydro-1',3',3'-trimethyl-6-nitrospiro[2H-1-benzopyran-2,2'-(2H)-indole] (SP) Oxford blue (OB) 2-Methoxycinnamic acid (MCA) 4-Hexadecyloxy-3-methyl cinnamic acid (4-MCA)

The photochromic LC systems investigated were not as noticeably responsive as the photothermal systems, but activation of the photochromic components using UV/white light was shown to have some effect on the mesophase nanostructure. The most significant effects were observed in the AZB, 4-AZB & SP systems and are reported here. The requirement to dissolve the dye in the lipid limited the amount of dye that could be incorporated due to limited lipo-solubility. Whilst observing x-ray collection, the application of light caused a disturbance in the intensities of peaks and then the system settled within the exposure period of ~19mins. After the systems reached equilibrium, it was observed that the application of white light caused a right shift in the peak positions of the v_2 phase, and some cases, the appearance of the H_2 peak.

The AZB and 4-AZB in PHYT systems are presented in the SAXS plots in figure 3. Grey symbols represent samples at thermal equilibrium, white symbols represent white light exposure and blue symbols represent UV light exposure. Each break represents ~10 min light exposure time. The lattice parameter and phase type are reported on the right side of the plots. Samples were kept in the dark until analysis. Azobenzenes are incorporated into a wide range of different materials in order to impart photoreversible control. Azobenzenes undergo a *trans* (relatively hydrophilic)-to-*cis* (relatively hydrophobic) isomerisation on exposure to UV light and reverts back to the *trans* isomer in the absence of light or on exposure to white light. Two azo additives were used, azobenzene and a long chain derivative 4-Decyloxy azobenzene (4-AZB). White light exposure of the PHYT+AZB system shifted the v_2 peaks to the right and UV exposure shifted peaks to the left. The longer chain azobenzene appears to have a different effect on the bulk nanostructure. On application of white light, the first H_2 peak appears as indicated by red arrows. A similar response to the PHYT+4-AZB was observed in a GMO + SP system as shown in figure 4. Exposure of the system to white light caused the appearance/growth of a H_2 peak, and on exposure to UV, the H_2 peak retracts. Spiropyrans undergo reversible ionisation into a charged merocyanine (relatively hydrophilic) form when exposed to UV light, and reverts back on exposure to white light (relatively hydrophobic).

The initial change in intensities may be due to a steric disturbance in the system caused by the photo-isomerisation of the additives. Once the systems are at equilibrium, the relative hydrophilicity of the photoisomer influences the phase structure; the more hydrophobic the species, the more negative the curvature. On exposure to white light, both the isomers of the SP and azo additives are relatively hydrophobic and so the v_2 peaks are shifted to the right and/or H_2 peaks appear, and on exposure to UV light, the additives are relatively hydrophilic and so the v_2 peaks shift to the left and/or H_2 peaks disappear. These observations are interesting, but a more acute system response is desired. The observed effect may have been hindered by a few things, firstly the long collection time (100 sec) required by the Hecus instrument may have prevented the visualisation of any subtle changes; and secondly the physicochemical features of the additive, namely limited solubility in the LC and the extent and length of time required for photoisomerisation. Further experiments will be focussed on developing a photochromic LC system which has a more defined response to light activation and also on using a resolute method to investigate the kinetics of photo-activation.

DATA

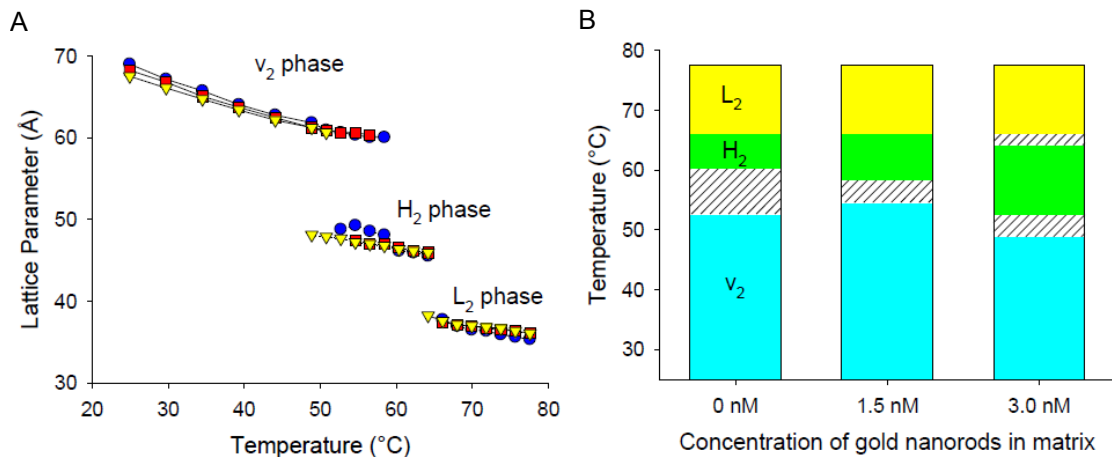


Figure 1 - Effect of gold nanorod concentration on equilibrium structure of the PHYT + water system with controlled temperature from SAXS experiments. Panel A illustrates the lattice parameter of the phytantriol + water system in the presence of 0 nM (●), 1.5 nM (■) or 3 nM (▼) GNRs. Panel B shows the influence of GNR concentration on the transition temperatures of the phytantriol + water system (blue region = v₂ phase, green = H₂, yellow = L₂, hashed regions are mixed phase).

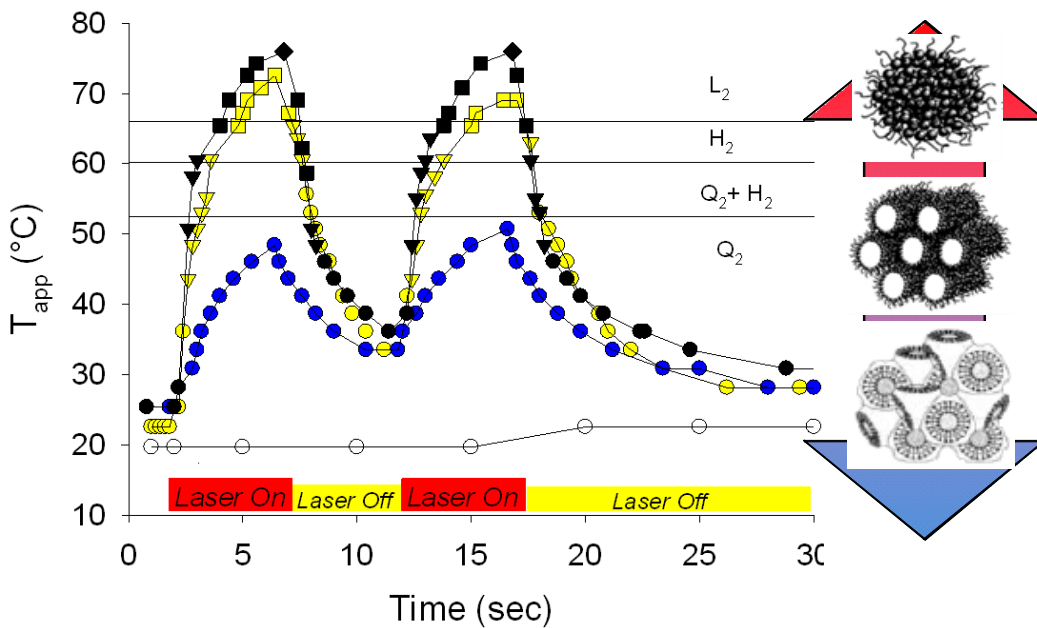


Figure 2 – The effect of laser irradiation on apparent temperature (T_{app}) of the phytantriol + water matrix with increasing GNR concentration. T_{app} was derived from lattice dimensions in kinetic synchrotron studies, using the calibration data from Figure 1. [GNR] = 0 nM white symbols, 0.3 nM blue symbols, 1.5 nM yellow symbols, 3 nM black symbols. Circles indicate v₂ phase, triangles indicate v₂ + H₂, squares indicate H₂ + L₂ and diamonds indicate L₂. The horizontal lines indicate the typical equilibrium phase boundaries derived for the data in Figure 1. The cartoon on the right indicates the type of phase structure present with increasing temperature (v₂, H₂ & L₂).

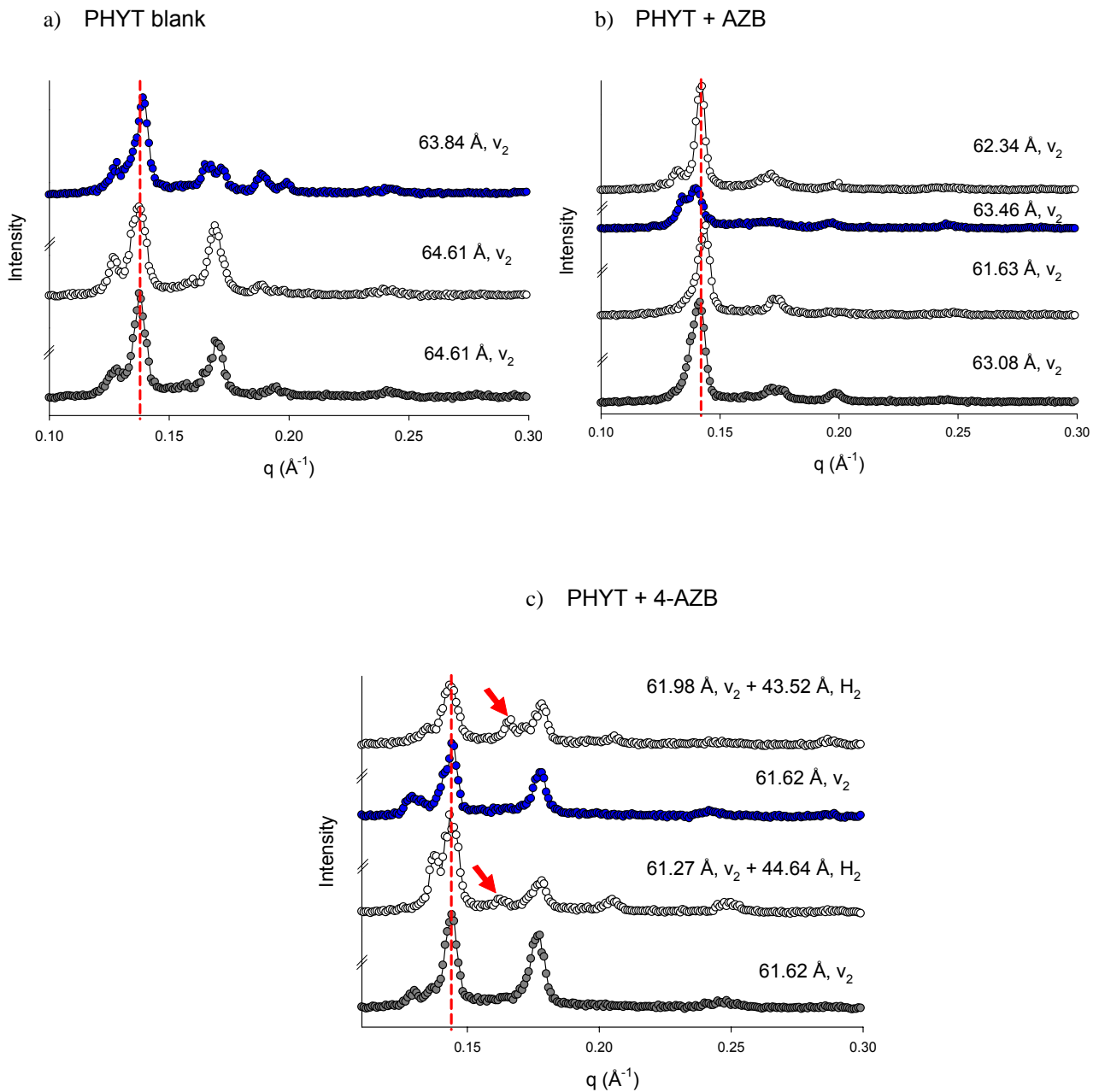
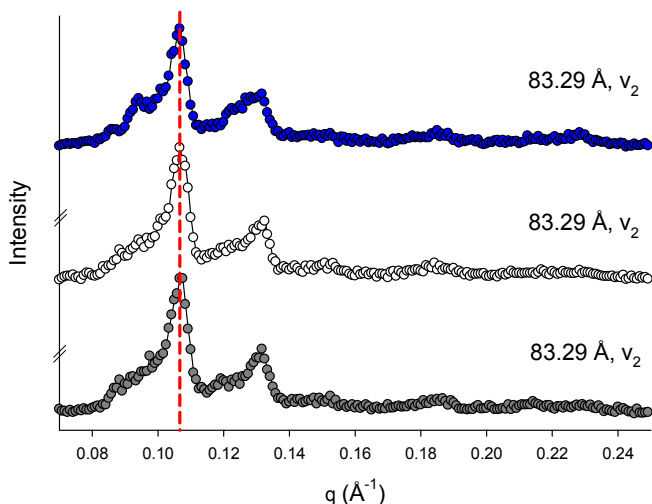


Figure 3 – SAXS plots of dynamic light exposure studies on PHYT azobenzene systems. Gray symbols represent samples at thermal equilibrium, white symbols represent white light exposure and blue symbols represent UV light exposure. Red dashed lines are present as a guide to observe shifts in peak positions. Red arrows indicate the emergence of a H_2 peak. Lattice parameters and phase types are reported on the right side.

a) GMO blank



b) GMO + SP

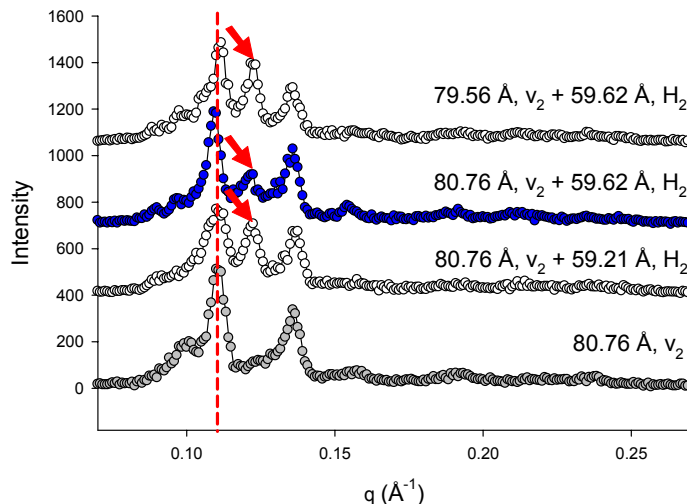



Figure 4 – SAXS plots of dynamic light exposure studies on GMO + SP systems. Gray symbols represent samples at thermal equilibrium, white symbols represent white light exposure and blue symbols represent UV light exposure. Red dashed lines are present as a guide to observe shifts in peak positions. Red arrows indicate the emergence of a H₂ peak. Lattice parameters and phase types are reported on the right side.

Signature of Investigator preparing the report for After signing this report please fax this page with your signature for our files	Proj: AINGRA09120 Date:
	2/2/2010

PUBLICATIONS / REPORTS arising as a result of your work.

Wye-Khay Fong, Ben J Boyd & Tracey Hanley; 2009; Photoresponsive additives in lyotropic liquid crystals; abstract in the 16th AINSE Conference on Nuclear and Complementary Techniques of Analysis; Lucas Heights; November 2009 ✓*

Wye-Khay Fong, Tracey Hanley, Benjamin Thierry, Nigel Kirby, Ben J Boyd; Submitted to Nano Letters 12th Jan 2010; Plasmonic nanorods provide control over nanostructure of self assembled drug delivery materials ✓

PhD STUDENTS

Wye-Khay Fong - Correlating the nanostructure of stimuli responsive liquid crystal systems with drug release behaviour in vitro and in vivo, September 2012 anticipated thesis submission