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IN FOCUS: NANOMEDICINE - ARTICLE

Alkylation of Spiropyran Moiety Provides Reversible Photo-Control over Nanostructured Soft Materials

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Abstract The purpose of this study was to create a light responsive nanostructured liquid crystalline matrix using a novel alkylated spiropyran photochromic molecule (spiropyran laurate, SPL) as a light activated drug delivery system. The liquid crystal matrix, prepared from phytantriol, responds reversibly to changes in photoisomerism of SPL on irradiation, switching between the bicontinuous cubic and the reversed hexagonal liquid crystal structures, a change previously shown to dramatically alter drug release rate. In contrast, the non-derivatized spiropyran and spirooxazine photochromic compounds do not sufficiently disrupt the matrix on isomerization to induce the phase change. Thus, novel alkylated spiropyran has the potential to be an effective agent for use in liquid crystalline systems for reversible 'on-demand' drug delivery applications.

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1 Introduction

Light responsive soft materials have been proposed for many bioapplications, including the development of 'on-demand' drug delivery systems [1]. Towards this goal, photochromic additives and photothermal nanoparticles have been used to impart photosensitivity into materials such as polymers and self assembly structures [2–5]. When exposed to specific wavelengths of UV light, photochromics can reversibly switch between two isomeric forms of the chemical species. This feature has lead to photochromic moieties, such as spiropyran and azobenzene, being incorporated into self assembled systems as a trigger for drug release [6–8].

Of particular interest is the photo-isomerization of spiropyrans, between the colorless, non-ionic spiro and the colored, charged merocyanine forms [9]. This family of photochromics has been well studied and been shown to induce changes in materials such as liquid crystal phase structure [10], light-induced reversible dissolution of SP-modified block copolypeptide micelles for drug release [8] and modifying the self-assembly of lipid and surfactant membranes [11–14]. Notably, lipid-based liquid crystal materials are receiving current interest as pulsatile active release systems as they can form thermodynamically stable nanostructures, which control the rate of drug release from the material [15–20]. They can also be rendered pH responsive by inclusion of ionizable lipids for selective release during oral drug delivery [21]. Sufficient disturbance in the lipid packing can cause a change in nanostructure and thus 'trigger' a change in drug release. Using small angle X-ray scattering (SAXS), we have previously

shown reversible control over the nanostructure using temperature as a stimulus, and consequent drug release rates from the liquid crystal matrix both in vitro and in vivo [22]. However, for some applications, direct heat is not practical and a non-invasive stimulus is necessary.

With the need for a non-invasive means of controlling drug release, the purpose of this study was to create and characterize a light responsive spiropyran-based liquid crystalline drug delivery system. We report the effect of irradiation of photochromic spiropyran-based dyes incorporated into liquid crystalline systems based on phytantriol (Fig. 1). The dyes are hypothesized to alter lipid packing on isomerization, inducing changes in nanostructure, and thereby could act to trigger drug release from liquid crystal matrices. Further we introduce a novel alkylated spiropyran derivative, spiropyran laurate (SPL), hypothesized to interact more strongly with the lipid matrix than non-derivatized spiropyran. The photochromics, illustrated in Fig. 1 (spiropyran (SP), spirooxazine (SOX) and SPL) were added to the liquid crystalline matrix and the effect of irradiation on nanostructure was determined using synchrotron SAXS.

2 UV Characterization of Spiropyran Laurate

The UV–visible spectral characteristics were determined for the novel SPL (reported in detail in the Supporting Information). The UV–visible scan of SPL in methanol (Fig. 2) shows that the molecule absorbs strongly at UV wavelengths. Exposure to UV light leads to the appearance of a peak at 540 nm, corresponding to the formation of the open merocyanine form of the SPL, in accordance with previously reported behavior for the non-alkylated

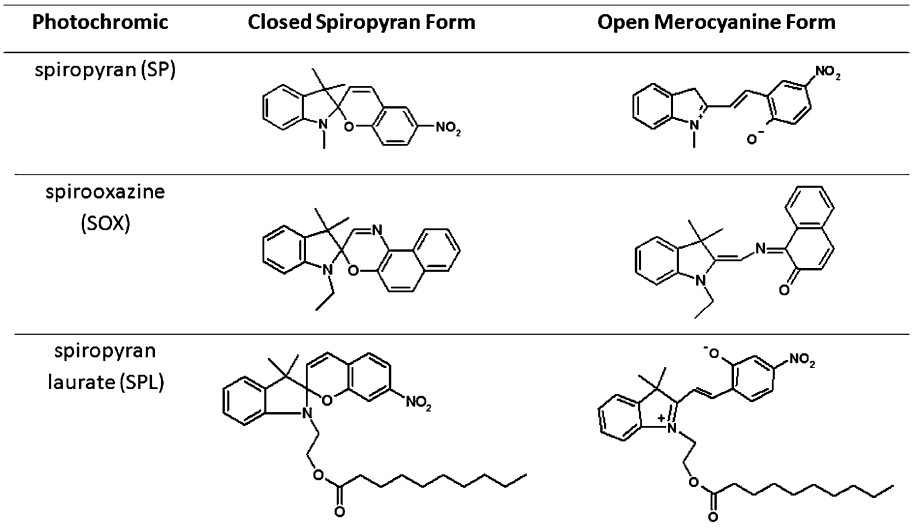


Fig. 1 Chemical structures of spiropyran laurate (SPL), spiropyran (SP) and spirooxazine (SOX)

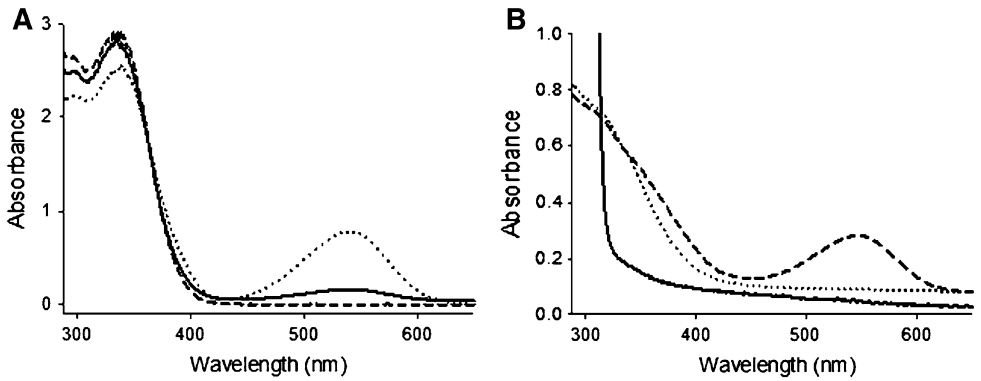
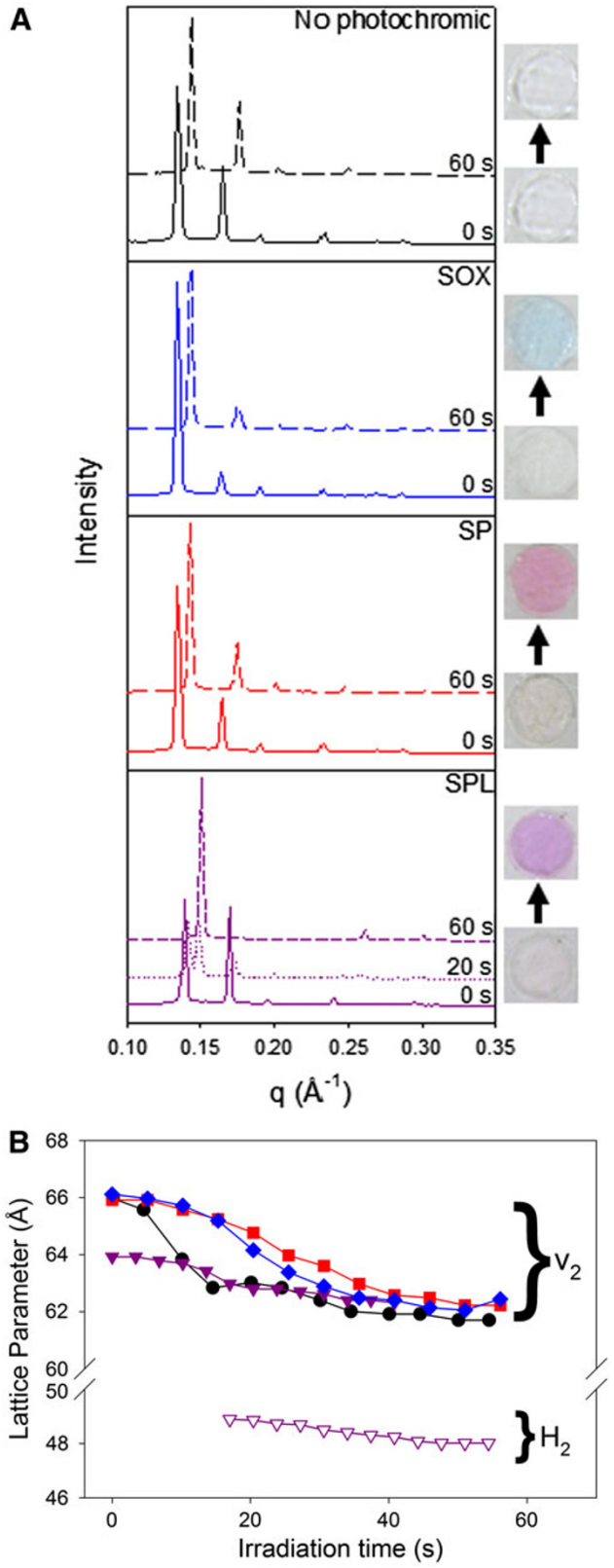


Fig. 2 **a** UV–visible spectra of SPL in methanol (1.87×10^{-3} M, 298 K) with curves corresponding to equilibrium condition without light (*continuous line*), after 10 min UV light exposure (*dashed line*) and after 10 min white light exposure (*dotted lines*). **b** UV–visible spectra of phytantriol cubic phase in excess water without photochromic (*continuous line*) and SPL in phytantriol cubic phase (2.55×10^{-4} M, 298 K) with curves corresponding to 10 min UV light exposure (*dashed line*) and after 10 min white light exposure (*dotted lines*). The peaks at 300–350 nm are assigned to the closed spiro form of the SPL and the peak at 540 nm assigned to the open merocyanine form of the SPL



◀ **Fig. 3** **a** SAXS scattering profiles for phytantriol–water liquid crystal matrices containing: (top → bottom) phytantriol only, 2% (w/w) SOX, 2% (w/w) SP, 2% (w/w) SPL. The matrices were exposed to 60 s of UV light (375 nm, 60 mW) and the mesophase structure followed over this time. The *insets* show the color change of the matrices from clear to *pinky-purple* (SP), *blue* (SOX) or *purple* (SPL), before and after UV exposure due to the transition from the clear spiropyran form to the colored merocyanine form (Fig. 1). **b** The lattice parameter over time of the matrices where *filled circles* phytantriol only, *filled squares* SP, *filled diamonds* SOX, *filled down triangles* SPL, *closed symbols* represent v_2 (Pn3m space group) and *open symbols*, H_2

spiropyran molecule [9, 23]. The absorbance for the merocyanine form when loaded into the transparent cubic phase is essentially identical (Fig. 2). The rate of opening and closing of the SPL spiropyran ring in methanol mirrors that of previously reported rate constants for SP, however, the observed rate constants (SI Table 1) indicate that in non-polar environments, the SPL preferentially adopts the closed spiropyran form due to the polarity of the solvent stabilizing the closed spiropyran form [24, 25]. Consequently, the spiropyran moiety opens in such a miniscule proportion in hexane that it is almost impossible to detect the rate constant for merocyanine ring closing. In comparison, when in a relatively polar environment such as methanol, the open merocyanine form is partially stabilized and at equilibrium both the closed spiropyran form and a small percentage of the open merocyanine form are both present. The phytantriol liquid crystal medium has a polarity in between that of methanol and hexane. As such, the SPL in phytantriol liquid crystal demonstrates a rate constant between the two solvents (Fig. 2b and Supplementary Information).

3 Effect of Photochromics on Nanostructure

Three structurally related photochromics added to a phytantriol-water liquid crystal matrix were compared in this study for their effectiveness in disrupting lipid packing on irradiation. Briefly, the photochromics, SP, SPL and SOX were pre-dissolved in phytantriol, and phosphate buffered saline (pH 7.4) was added to the lipid phase in a ratio of 1:1 (w:w) to ensure excess water conditions [10, 26, 27]. The samples were heated transiently to 70°C to enable vortex mixing three times, and left to equilibrate for 1 week at 25°C before irradiation experiments.

In the absence of irradiation, the liquid crystal structure was not affected significantly by the presence of the photochromic additives (Figure SI2). The structural changes for the phytantriol liquid crystal matrices containing 2% (w/w) of SP, SPL or SOX from the initial structure at $t = 0$ to that at 60 s of UV irradiation are compared in Fig. 3. (For clarity only the SAXS profiles at 0 and 60 s are illustrated; the full profiles are shown in Fig. SI 3 with 10 ms frames acquired every 2 s). The acquired SAXS patterns show that all matrices begin in the v_2 phase with

Pn3m spacegroup, as the peaks have spacing ratios of $\sqrt{2}$:$\sqrt{3}$:$\sqrt{4}$:$\sqrt{6}$:$\sqrt{8}$:$\sqrt{9}$… [28]. Over the 60 s of UV irradiation, the SP and SOX systems exhibited changes in lattice dimensions (Fig. 3b), indicative of some disruption of lipid packing. However, the effect was not sufficient to induce a phase transition. In contrast, the SPL system completely transitioned to the H_2 phase (peaks at spacing ratio 1:$\sqrt{3}$:$\sqrt{4}$) after 20 s of UV exposure. The lattice parameter for the H_2 phase (48–49 Å, Fig. 3b) is consistent with previous reports for the H_2 phase in phytantriol–water systems [10, 26, 27].

In order to exert the greatest effect on lipid packing, the photochromic molecules should align themselves in the lipid bilayer near the hydrophilic headgroups of the amphiphiles. We propose that the laurate tail on the amphiphilic spiropyran "anchors" the photochromic into this position, and so enhances the disruptive effect of the change in SPL structure on the lipid packing. In contrast, the charged merocyanine form of the SP has the potential to partition out of the lipid bilayer into the aqueous domain on ionization, thereby losing its ability to disrupt the lipid packing and nanostructure. The reason for the lack of effect on photoisomerisation of SOX is less clear. This small, hydrophobic molecule may preferentially reside in the hydrophobic regions at the intersection on the tails of the phytantriol, and hence its isomerization does not cause a substantial disruption to lipid packing. Future experiments are planned to confirm these hypotheses.

The light-induced phase transition of the SPL–phytantriol–water system was found to be reversible. Figure 4 shows the changes in scattering, indicative of changes in nanostructure of the matrix, with repeated UV exposure. The rate of reversion of the phase structure back to the original v_2 phase on cessation of UV exposure is anticipated to depend on the SPL molecular relaxation rate and the rate of transition of the liquid crystal phase back to the cubic phase. Both of these processes occurred in this system within seconds. The SPL preferentially adopts the spiro form in the liquid crystal matrix, evident from the value for k_{2obs} (SI Table 1) and so rapidly reverts back from the merocyanine form when the irradiation ceases. Thus, the rate of liquid crystal structural reversion depends mainly on the speed of phase reversion which has been shown to occur within seconds. The authors have recently reported a photo-responsive liquid crystal matrix doped with gold nanorods, capable of responding to near infrared light, where the plasmonic response of the gold nanorods elicits a strong, reversible photothermal response from the matrix [5]. The emergence of a non-equilibrium v_2 phase with Ia3d spacegroup during relaxation was also observed, in accordance with previous reports where supercooling and the existence of non-equilibrium structures on transition from H_2 to v_2 phases have been previously shown to occur in phytantriol liquid crystal systems [5, 29, 30].

In this study, the effect of irradiation of liquid crystal containing the photochromic dyes spiropyran, its monolaurate derivative and structurally similar spirooxazine were compared. On irradiation with UV light, the liquid crystal matrix containing the spiropyran laurate (SPL) induced changes in the nanostructure, whereas the non-alkylated

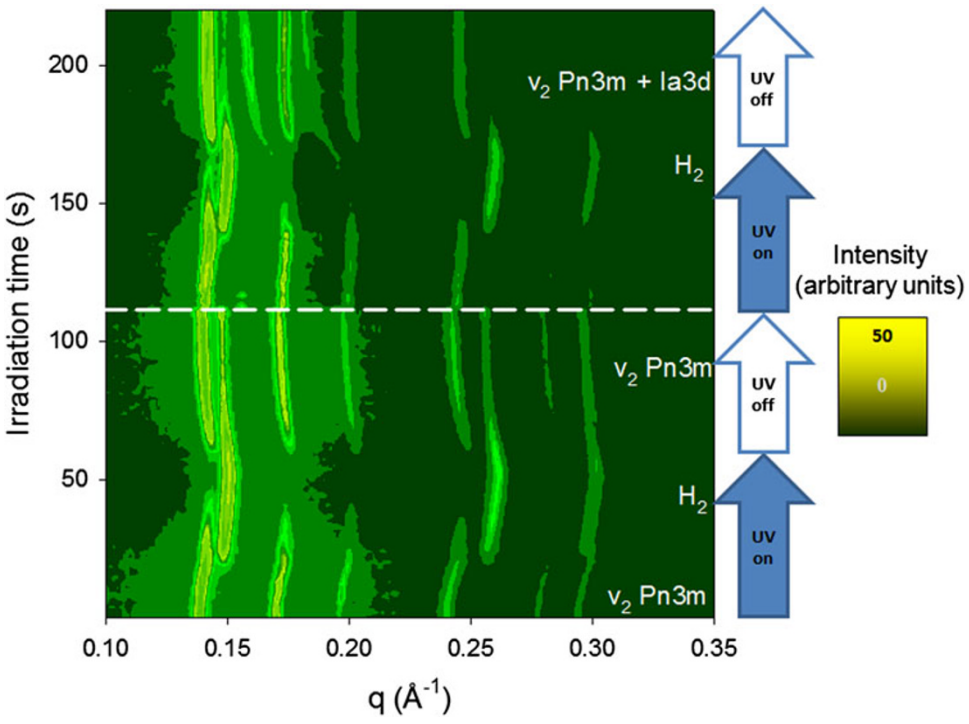


Fig. 4 Time resolved SAXS plot showing reversible phase transitions of the SPL-phytantriol liquid crystalline system. *Brighter shading* indicates greater scattered intensity. Samples were exposed to UV light (375 nm, 60 mW) two cycles of 60 on, 40 s off. The *white line* indicates the start of the second cycle and a change in sample position. The liquid crystalline phase transitions are annotated on the right

spiropyran and spirooxazine did not. Non-alkylated SP had little effect on structure, and is hypothesized to partition out of the nanostructure on ionization, resulting in little disruption to lipid packing. The UV response of the SPL–phytantriol matrix was also found to be reversible. It is anticipated that this approach can be applied to control changes in drug delivery rate from lyotropic liquid crystals, and hence provide novel, reversible, 'on-demand' drug delivery systems.

The application of these materials in drug delivery is anticipated to be via injection of an in situ 'gelling' lipid matrix. Administration of the matrix to e.g. subcutaneous tissue imbibes aqueous fluid forming the liquid crystalline structure in vivo. We have previously provided proof of concept for such a system responsive to temperature [22]. Penetration of UV radiation into tissues is obviously a limitation for such a system, however recent work in the polymer field has shown how photochromic spiropyran systems can be activated by NIR irradiation of UV emitting upconverting nanoparticles [31], providing a potential route for practical application of the materials described in this study which we are currently investigating.

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