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The integration of triggered drug delivery with real time quantification using FRET; creating a super 'smart' drug delivery system

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ABSTRACT

The ability to control drug release at a specific physiological target enables the possibility of an enhanced therapeutic effect with reduced off-target toxic side effects. The discipline of controlled drug release has grown to include most areas of medicine with examples in the literature of targeted drug delivery to the majority of organs within the human body. In addition, a variety of external stimuli used to meditate the drug release process have also been investigated. Nonetheless, the concurrent real time monitoring of drug release has not been widely studied. In this manuscript, we present a novel micellar drug delivery system that is not only capable of releasing its cargo when stimulated by light but also provides a real time analysis of the amount of cargo remaining. Controlled drug release from the delivery system was mediated by physicochemical changes of a spiropyran-merocyanine photochromic dyad, while drug quantification was enabled using a Förster Resonance Energy Transfer (FRET) relationship between the photochrome and a co-encapsulated BODIPY fluorophore. The percentage of drug released from the delivery system was significantly greater (24%) when exposed to light irradiation compared to an analogous control maintained in the dark (5%). Furthermore, the fluorescence read-out capability also enabled the drug-release process to be followed in living cells with a significantly reduced fluorescence emission observed for those cells incubated with the delivery system and exposed to light irradiation compared to control cells maintained in the dark. Combined, these results highlight the utility of this approach to theranostic drug delivery with the potential of light-triggered released together with a fluorescence read-out to enable quantification of the drug release process.

1. Introduction

In order for a drug to produce a therapeutic effect, it must not only reach the site of action but also have the correct physicochemical properties to allow it to be absorbed at an appropriate concentration. In the past few decades, smart drug delivery systems (DDS) have evolved to deliver an appropriate dose to meet the patients needs [1]. Delivering the drug at a controlled rate, triggered drug release and targeted drug delivery are some methods that have been extensively investigated. Some examples of such systems include the development of bio-pharmaceutical systems capable of interacting with intracellular components that respond as a direct result to environmental stimuli [2] and nanoparticles that specifically bind to tumour cells using receptor targeted systems [3,4]. Among these, triggered release plays a substantial role on controlling timing and location of drug release, since it can be induced by several external stimuli acting on the intracellular vehicles response [5]. Examples of stimuli used to facilitate drug release are temperature [6], pH [7], magnetic field [8], electric field [9], ultrasound [10], enzymatic activity [11] and light [12].

Light responsive drug release is an attractive mechanism because of the ability to control the spatial and temporal triggering of the release process [13]. Numerous examples of photochromic materials capable of transforming under the influence of activating radiation have been explored over recent years [14]. However, the photo activating ability of Spiropyran compounds was recognised as early as the1920s [15]. Spiropyran can undergo a reversible response to light and chemical stimulations. The closed ring stable state of Spiropyran (a) can be converted to its open form, Merocyanine (b), when irradiated with UV light, which is converted back into its original state when irradiated with visible light (Fig. 1).

This simple photochromic transformation has found many applications ranging from molecular sensors [16] to DNA-based logic

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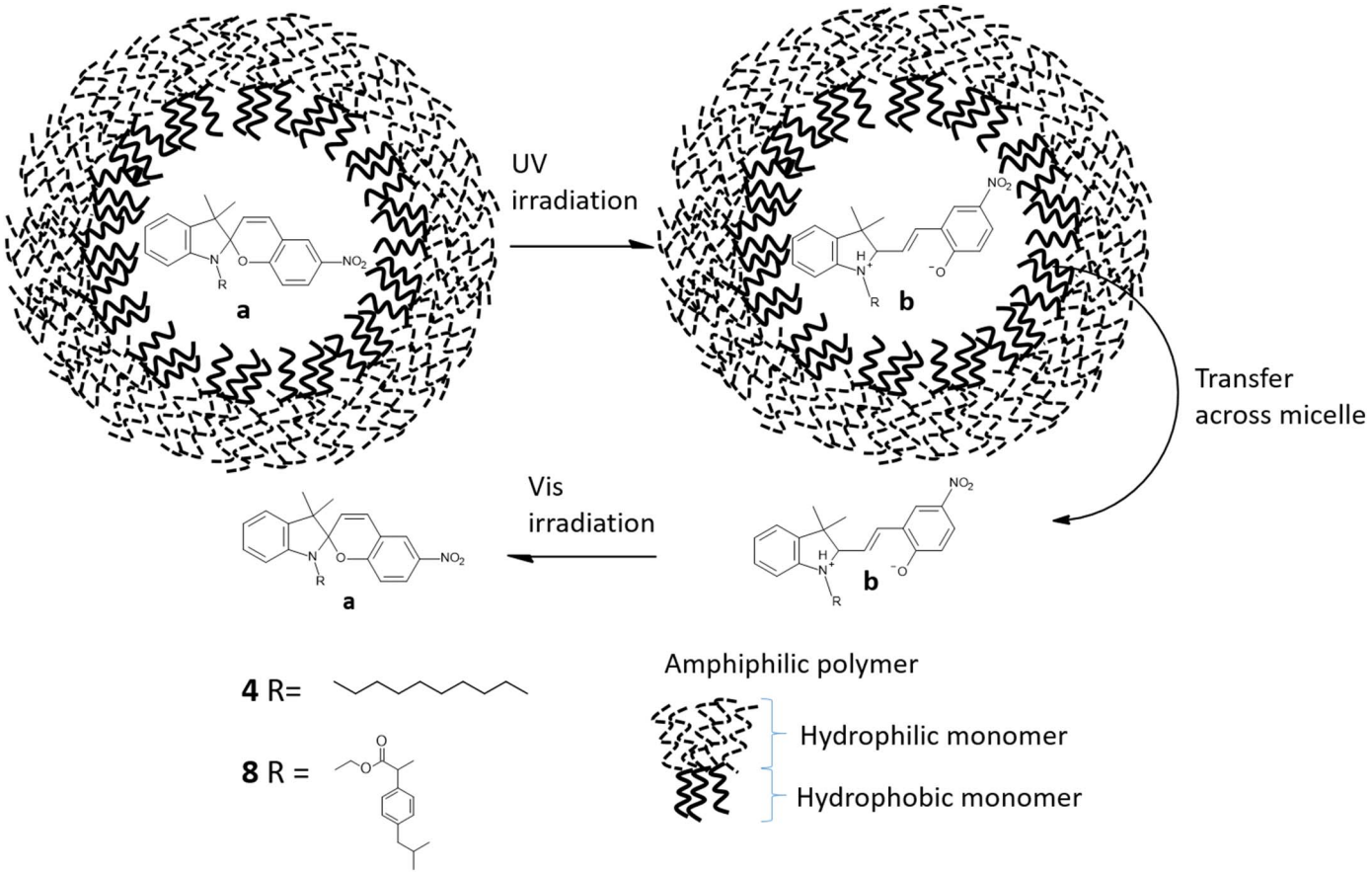

Fig. 1. Illustration of the phototranformation of spiropyran (**a**) to its merocyanine (**b**) counterpart and subsequent movement within the micellar environment before transformation back under visible irradiation.

operations [17] and bioimaging [18]. Here, we exploit differences in the hydrophobic/hydrophilic balance between the two isomers to mediate drug release from a micellar based delivery system. The spiropyran form, with its hydrophobic four-ring system preferentially favours a non-polar environment whereas its open ring zwitterionic merocyanine counterpart prefers a more hydrophilic environment.

In addition to the triggered delivery mechanism there are also a number of considerations to make when selecting the type of DDS. More than 40% of newly discovered drugs have little or no aqueous solubility (as determined by the Biopharmaceutical Classification System): 90% of drugs approved since 1995 have poor aqueous solubility, poor permeability or both [19]. The delivery of hydrophobic drugs can be achieved in a number of ways. For example, a pro drug of the active compound may be prepared to catabolize to the original drug. Alternatively, a specific functional group can be altered to create a synthetic analogue with more appropriate hydrophilicity, or the compound may be formulated in such a way as to enable delivery by enteric coating for oral delivery or by an alternative method such as rectal or intravenous administration. All of these approaches have proven successful in delivering hydrophobic compounds. However, these approaches can often lead to enhanced first pass effects and thus the requirement for higher dosage or enhanced expense or, depending on the dosage form, poor patient compliance.

An alternative and successful method for the delivery of hydrophobic drugs is the use of polymeric drug delivery systems. These DDSs can be formulated as, micelles [20], liposomes [21], nanofibers [22], dendrimers [23], colloids [24] or carbon nanotubes [25] with the majority of them falling into the category of nanoparticle drug delivery vehicles. It has been suggested that the polymeric nano carriers can become concentrated preferentially in tumors, inflammatory sites, and at antigen sampling sites by virtue of the enhanced permeability and retention (EPR) effect of the vasculature [26]. Once accumulated at the site, these polymeric drug delivery vehicles can act as a drug depot, providing a source of API to be released as and when required. This leads to enhanced bioavailability, sustained/controlled release and decreased toxicity caused by potential burst release of the API. There are numerous examples where polymeric compounds are shown to enhance drug delivery [27]. Among these systems Polyethylene glycol (PEG) is frequently used as a polymeric component. We have previously developed a PEG-micellar DDS and determined the size of our PEG copolymers to have an average hydrodynamic diameter of 26 nm [18]. As a direct result of their size, these micelles can navigate through the endothelium in inflammatory sites, epithelium tumors or penetrate micro capillaries, allowing for uptake by a variety of cell types. We have previously shown these micelles to efficiently cross the cell membrane of Chinese hamster ovarian cells and distribute themselves in the cytosol.

Finally, the ability to quantitatively monitor the amount of drug release, from a DDS in real time using a simple but effective approach is an essential companion in the advance towards second-generation health care. To this end, there have been a number of examples where mesoporous silica nanoparticles (MSN) have been used as a cage for drug delivery with the drug co-incorporated alongside a photochromic compound [28], an oligonucleotide containing a recognition element [29] or a redox active FRET pair [30] so that the system operates like a molecular valve. In each case, the drug was prevented from exiting the pores of the NP due to the large bulky groups surrounding the MSN. On application of external stimuli, the outer layer (valve) was disrupted and the inner cargo released from the MSN. In addition to the triggered release, a Förster Resonance Energy Transfer (FRET) mechanism was