Chemistry | Lancaster University

Light-responsive polymer-based drug delivery systems

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Abstract

Classical therapy protocols have many limitations due to the molecular characteristics of the active pharmaceutical ingredient (API), and pharmaceutical/biomedical engineers and scientists seek to improve them. There is a continual market need for improved drug delivery systems (DDSs). The use of smart drug delivery systems (SDDSs) capable of the controlled release of drugs at specific locations/times is a very exciting area of research pursued by researchers in academia and industry. The main purpose of SDDSs is to improve the pharmacological activities of the APIs administered. The formulations, approaches and technologies that enable the introduction and movement of pharmaceutical substances in the body and enhance their efficacy and safety are of key importance in SDDSs. The use of polymer-based DDSs will be discussed and an overview of the chemical structures of those polymers will offer an understanding of the mechanisms of drug release, stimuli essential for the drug release and the uses and applications of light responsive polymers.

The main aim of the research project is the synthesis of biocompatible light responsive polymers for drug delivery.

The first chapter of results in this thesis will illustrate the preparation and characterisation of photoswitchable bioconjugates composed of the hydrophilic natural linear polysaccharide hyaluronic acid (HA) with the hydrophobic 2-diazo-1,2-napthoquinone (DNQ) conjugated to the backbone of the HA. These bioconjugates might offer enhanced therapeutic effects *via* improving the solubility of poorly water soluble drugs (e.g. doxorubicin, Cabozantinib, Nintedanib, Curcumin, Paclitaxel), pharmacokinetics, clearance, and potential to target cancer stem cells that overexpress cluster of differentiation 44 (CD44). Moreover, the HA-DNQ bioconjugates may assemble into micelle-like structures because of their inherently surfactant-like properties. Interestingly, the HA-DNA bioconjugates are photoresponsive because the DNQ can undergo a UV-induced Wolff rearrangement reaction rendering it hydrophilic, and therefore the micelle-like structures are potentially able to undergo triggered disassembly in response to the application of light. The loading and controlled release of dyes from the HA-DNQ micelle-like structures was studied using various dyes as model drugs.

The second chapter of results in this thesis involves the synthesis and characterisation of photocleavable cross-linkers to prepare photoactive polyethyleneglycol (PEG)-based hydrogels with varying quantities of photolabile crosslinkers; their properties were compared to non-photoactive PEG-based hydrogels with equivalent quantities of non-photolabile crosslinkers. In addition, photocleavable PEG derivatives (PEG-ONB-PEG) were also generated.

The last chapter of results in this thesis deals with a new compound that was synthesised in Dr John Hardy's lab by other members of the team (a PhD student Mark Ashton and an MSc student Alex Davey). The results from this chapter include light-sensitive changes to the PEG-hemin conjugates, optionally with a clinically relevant anti-inflammatory drug (dexamethasone 21-phosphate disodium).

Keywords

Smart drug delivery systems; Drug delivery vehicles; Cancer; Polymer based drug delivery; Photodynamic therapy; Polymeric micelles; Hydrogel with low-molecule-weight; and Light responsive polymer.

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List of Abbreviations

API	Active Pharmaceutical Ingredient
CD44	Cluster of Differentiation 44
DDS	Drug delivery systems
DI	Deionised water
DIC	N,N'-Diisopropylcarbodiimide
DLS	Dynamic light scattering
DMF	Dimethylformamide
DMP	Dexamethasone 21-phosphate disodium
DNQ	1,2-naphthoquinone-2-diazide4-sulfonyl chloride
DS	Degree of substitution
FTIR	Fourier-transform infrared spectroscopy
HA	Hyaluronic acid
H-PEO	Hemin-polyethylene oxide
NIR	Near-infrared spectroscopy
NIPAAM	*N*-isopropylacrylamide
NMR	Nuclear magnetic resonance
PBS	Phosphate-buffered saline
PDT	Photodynamic therapy
PEG	Polyethylene glycol
PEGDA	Poly ethylene (glycol)diacrylates
PEGDMA	Poly ethylene (glycol)dimethacrylates
PEO	Polyethylene oxide
PMs	Polymeric micelles
PS	Photosensitizer
SDDS	Smart drug delivery systems
TLC	Thin layer chromatography
UV	Ultraviolet

Introduction:

Many chronic diseases do not respond to conventional treatments. Classical therapy has many limitations due to inappropriate molecular characteristics which may lead to poor bioavailability, short half-life and physiochemical instability.[1] Consequently, both pharmaceutical and biomedical engineers/scientists seek to improve the effectiveness of therapeutics. The techniques and methods which are used in the development of novel drug delivery systems (DDSs) have progressed significantly since Robert Langer's pioneering research on biodegradable polymers for drug delivery to yield smart systems, capable of precisely targeted delivery to cells, tissues, or organs with minimal damage to healthy tissues. As a result, efforts to improve the delivery of medications through the use of smart drug delivery systems (SDDSs) are a hot topic in the scientific and patent literature. Precisely delivered drugs have the potential to be applied effectively to treat many serious illnesses like cancer tumours and neurodegenerative diseases.[2] These modern techniques may significantly increase the effectiveness of medicines and reduce their side effects. The main focus of research on SDDSs is to improve the pharmacological properties of drugs/formulations, approaches and technologies that enable the introduction and movement of pharmaceutical substances in the body safely.[3] SDDSs are mostly based on polymers that are sensitive to stimuli such as temperature, pH, light intensity, electric fields, magnetic fields and ultrasound which change their physiochemical properties such as functionality, colour and structure.[1,4,5] These compounds are "smart" because they respond to the stimuli and can potentially return to their original state after removing the stimuli.[1]

Polymer based drug delivery systems

Polymer design is complex; to achieve the required chemical, mechanical and biological functions of the polymer a thorough understanding of the bulk/surface properties of the polymer. The polymer-based drug delivery systems the polymers should either be capable of degradation under biologically relevant conditions to enable the disassembly of fragments of the polymer,[6] or to be small enough to enable renal elimination (<5.5 nm).[7,8,9,10]

Light-responsive polymers are being investigated for controlled release drug delivery and for simultaneous diagnosis and treatment (i.e. theranostics) of a variety of diseases, particularly, cancer. Cancers are complex and serious diseases that threatens people's lives. Cancer cells are hostile and destructive and result in one of the most serious mortality causes among people after cardiovascular diseases.[11] Therefore a significant amount of research focuses on finding effective and safe therapeutic strategies to eliminate cancerous cells.[11] The main challenge associated with traditional treatment methods of cancer is the difficulty of distinguishing between healthy and cancerous tissues particularly during surgery.[11] To achieve the solution of this challenge, researchers are applying the following techniques.

Photodynamic therapy (PDT)

The concept of photodynamic therapy emerged in 1900 when Oscar Raab, a medical student who worked in Germany, observed that some micro-organisms e.g. *Paramecia* might be killed with certain dyes when exposed to light in the presence of oxygen.[12] Haematoporphyrin was the first discovered water-soluble photosensitizer (PS) mixture of porphyrins by Thomas Dougherty and co-workers.[12] Despite the disadvantages related to photofrin (Porfimer sodium), including skin allergy, which may extend for a long time, may cause patient anxiety,[12] it's still frequently used in clinical practice although its drawbacks. The high absorbance of light by tissues, makes it challenging to use for deep tissues, particularly for bulky tumours which require minimally invasive light sources to treat them.[12] Hence, photodynamic therapy of cancers is typically carried out on skin cancers, and chemists have developed PSs.[12] Photodynamic therapy enables the treatment of cancer by choosing the appropriate wavelength of light triggers the generation of reactive oxygen species that kill the cancer cells. Photosensitiser substances, light intensity and oxygen atoms are the three essential components of photodynamic therapy. The limitation of this treatment approach represented by the amount of reactive oxygen species generated.[13]

Principles of photodynamic therapy

In the ground state, the two electrons exit in opposite spins (↓↑) that means the PS group is a singlet. When absorbing a photon with suitable quantum energy, one of these electrons will be excited into an orbital with higher-energy (illustrated by Jablonski's diagram, **Figure 1**).[12] Due to the unstable singlet excited-state of PS, the excess energy will be lost by the emission of light or internal conversion via heat production.[12] To produce parallel spins with a more stable excited triplet state, the excited singlet state of PS group may undergo a process defined as 'intersystem crossing'.[12] Subsequently, PS molecules may decay back to the ground state from the triplet-state by emitting a photon by phosphorescence. According to the quantum selection rules, this is a 'forbidden process'. Therefore, the excited singlet with a lifetime of only nanoseconds is much less stable than the triplet state having sufficient lifetime.[12] This allows transferring its energy by colliding with molecular oxygen (O_2), which is unique in being a molecular triplet in its ground state. This energy-transfer step will lead to the generation of singlet oxygen ($^1O_2$), a reaction known as a Type II photochemical process.[12] This may also occur through type I photochemical process whereby the PS in exciting state cab undergoes the reaction of electron transfer, that ultimately forms reactive oxygen species (ROS).[12] In this approach, the radical cation or anion can be formed via either acquisition or donation of an electron, then this radical anion reacts with oxygen atoms to produce the superoxide radical anion ($O_2^{\bullet-}$).[12] Hydrogen peroxide (H_2O_2) is formed by dismutation one-electron of $O_2^{\bullet-}$.[12] This process can also undergo another reduction by losing one-electron to create potent oxidant hydroxyl radical ($HO^{\bullet}$).[12] The mechanism of generation ROS via Type

I chemistry is mechanistically more complicated than via Type II, and most probably in the field of anti-cancer PDT, most PSs function via a Type II mechanism.[12]

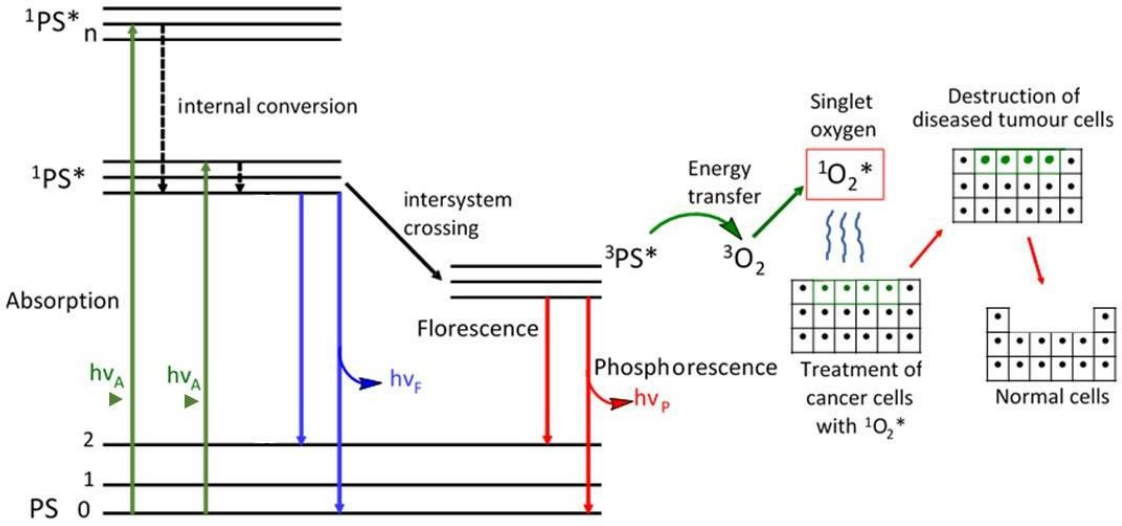

Figure 1. A Jablonski diagram that illustrates the electronic states of a molecule and the transitions between them.

Properties of PSs

PDT depends on non-toxic photosensitizers, which are activated by light absorption. When photosensitive groups absorb the incident light, the electrons will be excited and generate a reactive oxygen species, which leads to the destruction of the tumour cells.[12] In the clinic, most of the photosensitizers which been used for photodynamic therapy of cancer are based on the tetrapyrrole backbone (i.e. porphyrin derivatives), which accumulate in tumour tissues and absorb light wavelength ranges from 600 to 800 nm.[12,13,14]

Tetrapyrrole backbones are similar to that contained in the protoporphyrin group in haemoglobin.[15] There is a direct correlation between the wavelength and the depth of penetration where the depth of penetration increases with increasing the wavelength. The region of peak absorption should be between 650 and 800 nm since the region with wavelengths more than 800 nm cannot provide enough energy to excite oxygen atoms to their singlet state.[12] PSs should have a fundamental triplet quantum yield to produce a good ROS upon exposure to irradiation.[12] The ideal PS group should be made with good quality material that is pure, clean, and non-toxic with reasonable price as well as stable during storage.[12] To minimise the side effects that may be associated with phototoxicity, PSs should ideally have a relatively swift clearance from healthy cells and tissues.[12]

Light Sources

The common three wavelength ranges that are used frequently in cancer treatment, these are the ultraviolet (UV) (<400 nm), visible (400–700 nm), and long wavelength NIR region (700–1000 nm).[11,16] The wavelength, exposure time and quantity of light play an important role in treatment.[17] Ultraviolet irradiation is more widely used than other types of therapy in the treatment of skin cancer.[17] In general, light wavelengths of less than 700 nm are used for topical therapy applied to the external tissues (e.g. skin, eyes or mucosa).[17] This wavelength is relatively limited in therapy due to poor penetration of tissues (i.e. deeper than 1 cm) because of endogenous absorption and dispersion of the light by biomolecules (such as lipids, water and haemoglobin).[5,17] Other drawbacks of UV irradiation include causing damage to tissues that may be exposed to it.[17] By comparison, the wavelength range from 700 to 900 nm has the ability to penetrate deeper than UV radiation, therefore, NIR radiation has potential for therapeutic use for deep tissue.[17] Moreover, NIR radiation has the potential to deliver drugs to specific cells and tissues.[5] In general, photothermal, photo-isomerization and photochemical are three main mechanisms of light-based drug delivery systems.

Photothermal

Upon photoexcitation some materials can generate heat to influence thermally responsive components of SDDSs.[18] This approach involves two main components: the chromophore (that defines the transformation of light energy into thermal energy) and the thermally labile substances that respond to heat changes.[18] Both gold nanoparticles and poly-*N*-isopropylacrylamide (polyNiPAAm) hydrogels represent good examples for photothermal materials for delivery systems because they are absorbed rapidly in the NIR region of the spectrum. In polyNiPAAm hydrogels, temperature induced changes in the hydration of the polymer backbone result in a lower critical solution temperature. Above the lower critical solution temperature, polyNiPAAm goes from a swollen hydrogel to a globular state – expelling water (and dissolved drug(s)) from the dehydrated network.[18] The temperature at which this transition occurs can be tailored to physiological temperatures by controlling the hydrophilic/hydrophobic balance with the addition of co-monomers to the polymer network.[18] The long-term impact of gold nanoparticles in vivo is unknown and is a subject of ongoing studies, as is the availability of other thermally responsive substances to act as triggers.[18]

Photo-isomerization

Polymers containing moieties capable of reversible conformational changes after exposure to UV and/or visible light can be used for drug delivery.[18] An important example of moieties capable of undergoing such transitions are azobenzene derivatives, which consist of two phenyl groups joined by N=N bonds converting from trans to cis upon UV irradiation. Such conformational rearrangements of molecules can be used to create a “valve” that can ‘turn-on/turn-off’ drug release with good temporal resolution.[18] A significant problem of azobenzene derivatives is their potential

for irreversible decomposition leading to the formation of nitrobenzene and associated toxicity.[18]

Photochemical

This method can release the encapsulated cargo after exposure to light irradiation that breaks covalent bonds.[18] Following exposure to UV irradiation, ortho-nitrobenzyl (2-nitrobenzyl) irreversibly yields free carboxylic acids (COOH) and 2-nitrosobenzaldehydes. Currently, photo-responsive systems based on NIR radiation, which includes two-photon and up-converting nanoparticles are under investigation to replace the use of UV and visible light.[18]

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A Mini-Review on Polymeric Micelles for Drug Delivery and Cancer Cells Targeting

Abstract:

Polymeric micelles (PMs) are formed from an amphiphilic block that may be appropriate for loading poorly water-soluble drugs (e.g. anticancer drugs). PMs may be employed for directly targeting toward tumour sites by active or passive mechanisms. Delivering poorly hydrophobic anticancer drug to target sites by permeability can be potentially promising by using PMs. Several properties of PMs, including plasma stability, potential nanoscale size, longevity in vivo and high capacity for loading drugs allow PMs to have potential for targeting cancer cells while minimising damage to normal /healthycells. Promoting accumulation of drugs in cancer cells can be achieved by using stimuli-responsive nanocarriers for the development of SDDS, which respond to the application of an external or internal stimulus (such as temperature, light, pH or ultrasound).

Keywords: polymeric micelles; nanocarriers; light-responsive; drug delivery.

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1. Introduction

Conventional chemotherapy has many issues such as poor bioavailability, low therapeutic index, adverse side effects, as well as high-dose requirements; many advanced DDSs have been developed to address the poor release of drugs at the desired sites in a spatially controlled manner and reduce the dosage frequency, while reducing the adverse side effects and maintaining the drug concentration in targeted sites for a longer period of time.[1,2] Modern drug carriers include: nanoparticulate and nanotechnology-based delivery systems, hydrogels, polymer-conjugated drugs, and others.[1] In the area of cancer diagnosis and therapy, nanomedicines that are used as drug delivery vehicles are illustrated a significant development since they have been introduced in medical practices due to their unique biological properties involved such as the size of particles ranges from 1 to 100 nm, and appropriate surface area to volume ratio, which gives them opportunities to absorb, bind, and carry the anticancer agent.[1] Those nanocarriers can be divided into two major classes designed for targeted or non-targeted medication delivery; the first class of carriers is based on organic molecules such as polymers, liposomes, lipids, dendrimers and micelles as drug carriers, while the second class is based on inorganic materials.[1] DDS carriers based on polymers with dynamic characteristics have potential to significantly improve therapeutic efficacy and reduced side effects by responding to an appropriate stimulus, and such technologies have great potential for impact in the clinic.[1]

Most potent anticancer drugs are accompanied by poor water solubility (such as doxorubicin, paclitaxel, docetaxel, campothecin, etoposide),[3] due to having a lipophilic group for receptor admission and membrane permeability, which becomes the major obstacle for their therapeutic application.[4,5] To address this challenge, polymeric

micelles (PMs) acting as an efficient nanocarriers for drugs and pharmaceuticals offer significant potential for increasing drug delivery to potential therapeutic sites rather than delivery to healthy cells.[4,5] PMs are nanocarriers formed by polymers that self-assemble in aqueous media into assemblies with sizes typically ranging from 10 to 100 nm. PMs have been employed to deliver a variety of poorly soluble anticancer agents. Early in the 1990s they were used in the clinic, PMs were first introduced as cancer drug delivery systems by the Kataoka group who conjugated doxorubicin to copolymer micelles.[6]

2. Structure of polymeric micelles (PMs)

PMs are composed of amphiphilic polymers which often consist of two types of monomeric groups differing in their solubility.[4,5] These units assemble into a hydrophobic as inner core and a hydrophilic outer shell or corona (**Figure 1**). The inner hydrophobic core is the non-polar part of the block which encapsulates the poorly water-soluble drug, whereas the outer block of the amphiphilic is hydrophilic and protects drugs from the aqueous environment (e.g. enzymes that might degrade the drug/polymer). Polymers with appropriate structures may exist as unimers (unimolecular micelles), it is also possible to form non-micellar structures (gels, etc.).[4]

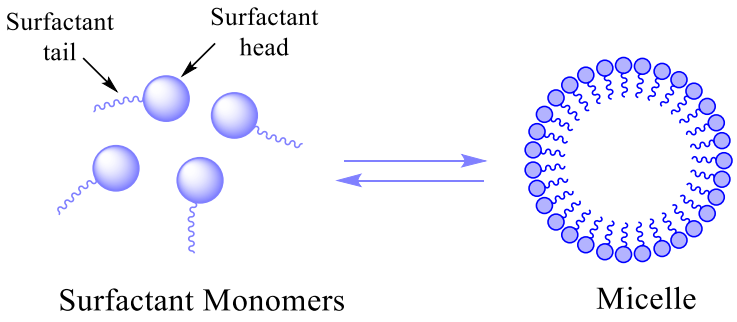


Figure 1. Micelle formation from amphiphilic block in aqueous solution (with hydrophobic tails and hydrophilic head groups).

The degree of self-assembly typically depends on the concentration of the polymer chain, the drug properties and the composition and the mass of the copolymer backbone.[5,7] According to the molecular weight of the copolymers, micelles can differ in structures including spherical, cylindrical, star and planar-shaped (**Figure 2**).

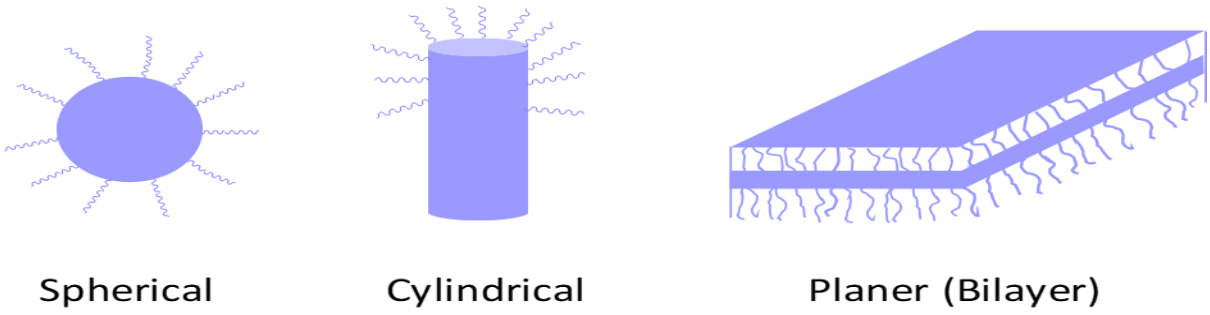


Figure 2. Different forms of micelles and assemblies for surfactants.

Several classes of polymeric micelles may be fabricated to deliver medication at the target site.[4,5,6,8,9] The common classes of polymers that have been utilised to make

polymeric micelles are block and graft copolymers.[6] Block copolymers are arranged in linear or radial arrangements (star structures), composed of at least two polymer units that differ in physical and chemical properties (e.g. charge or polarity).[6,8] Accordingly, the architecture of copolymer can be categorised to di-block (AB) (potentially hydrophilic-hydrophobic), tri-block (ABA) (potentially hydrophilic-hydrophobic-hydrophilic) or multi-block (ABC).[6,8,9,10] Graft copolymers are synthesised when the blocks are grafted to the backbone of other blocks.[5,6]

Blocks carrying a charge can produce stable complexes in water together with oppositely charged micelles, e.g. complex polyions or polyelectrolyte micelles.[11] Another advantage is the hydrophilic/hydrophobic balance in a graft copolymer structure can simply be controlled by altering the relative grafting density between the grafted block and the backbone.[5]

2.1. The hydrophilic blocks

The charge, length and density of the surfactant are responsible for protecting micelles and determining their hydrophilicity.[4] Polyethylene glycol and polyethylene oxide are frequently used to form the hydrophilic component of polymers.[9] The hydrophilic/hydrophobic mass ratio has a significant effect on the synthesis of amphiphilic copolymers or forming aggregates.[9] It is clear that if the mass of block of hydrophilic is too great, the copolymer will be a unimer in aqueous solution, while if the mass of hydrophobic block is too much, the polymer may not be soluble and therefore micelles might not be formed.[4,9,12.13] If however, both masses are similar or close to each other, conventional micelles can be formed.[4]

2.2. The Hydrophobic blocks

Determining characteristics, loading and releasing performance of micelles (e.g. depicted in **Figure 3**) depends on the nature, physical and chemical properties of the hydrophobic core blocks.[14] Hydrophobic blocks should be biodegradable (e.g. polyesters), nontoxic, with high loading capacity as well as good compatibility between the hydrophobic block and the incorporated drug[4,9,12,14]. When their hydrophobicity increases, the thermodynamic stability of micelles is improved.[14] Numerous researchers have indicated that the length of hydrophilic blocks has a considerable effect on the size of micelle and efficiency of loading.[14]

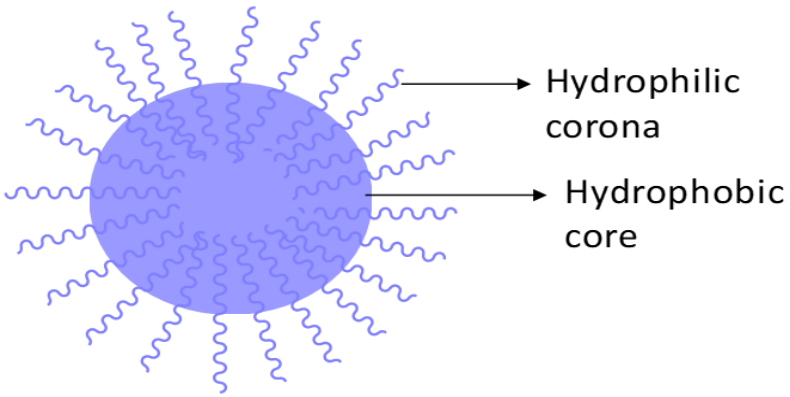


Figure 3. Cartoon depicting the structure of polymeric micelles.

3. Characteristic of polymeric micelles

There are two main factors that influence micelles stability: i) The micelle size and the critical micelle concentration (CMC). [13,14] These parameters are affected by the physicochemical properties and polymer characteristics such as molecular weight in addition to the composition of hydrophobic and hydrophilic blocks e.g. length, polarity, hydrophobicity, and degree of crystallinity; ii) The solubilisation enhancement of poorly soluble drugs in the core block of micelles which is strongly based on the size of micelle, size distribution and strength of the interaction between the hydrophobic blocks and drug molecules.[14] This factor reflects the speed/rate of the drug release.

3.1. The molecular weight

To avoid swift glomerular filtration within the human body, the molecular weight of polymeric micelles should more than a threshold value of 42–50 kDa for water-soluble synthetic polymers if (and only if) they are biodegradable.[14] IReports suggested that the micelle size and the molecular weight can be increased until a specific threshold, after which, micellization cannot occur due to the hydrophobic blocks being too large.[14]

3.2. Micelles structure

The structural characterisation of the block copolymer provides information about the size, size distribution, micelles density, microstructure, dimensions of the hydrophobic blocks and the CMC.[6,8,13] Microscops, dynamic and static light scattering (DLS and SLS), respectively, can be utilised to determine size and size distribution.[6,8]

3.3. Stability of polymeric micelles

The stability of a micellar system can be understood by two major parameters, including thermodynamic and kinetic.[13] The thermodynamic stability is directly associated with CMC which is mainly based on the hydrophobic property of the molecule. Therefore, polymers with high hydrophobic contents exhibit lower stability compared to polymers with high hydrophilic contents.[13]

Literature data demonstrates that increasing the length of a hydrophobic core block of the polymer while maintaining an identical content (number, structure) of hydrophilic branches results in decreasing CMC while the stability is improved. Those polymers which have low CMC can retain their stability even in very dilute forms within the blood circulatory system.[13] In contrast to thermodynamic stability, kinetic stability is related to the dissociation of polymeric micelles into single chain at concentration below their CMC values.[13] Fundamentally, the physical state of the core, the size of core block, quantity of solvent inside the core and the hydrophobic/hydrophilic balance are the most important parameters governing kinetic stability.[13] Covalent crosslinking is a considerable factor of increasing the polymeric micelles stability.[13] This class of stability can be achieved by photo-and thermal-induced polymerisation.[13] In addition, the kinetic stability can be achieved by the modification of micelle hydrophobic core with structures that can form intra-micellar structures, covalent crosslinking (potentially via click chemistry, which provides polymeric micelles with sufficient stability)[13] and/or supramolecular interactions.

3.4. Critical micelle concentration (CMC)

Above the CMC amphiphilic polymers can associate to form aggregates known as micelles (**Figure 4, Table 1**).[8] Numerous techniques can be utilised to determine CMC, including changes in physiochemical properties, fluorescence, or by measuring the surface tension. The CMC can also be determined by using dye solubilisation (e.g. using pyrene, Nile Red and 1,6-diphenyl-1,3,5-hexatrine).[6] These are non-polar molecules which change their fluorescent properties (e.g. vibrational changes and shifts in the emission and excitation spectrum, respectively).[13]

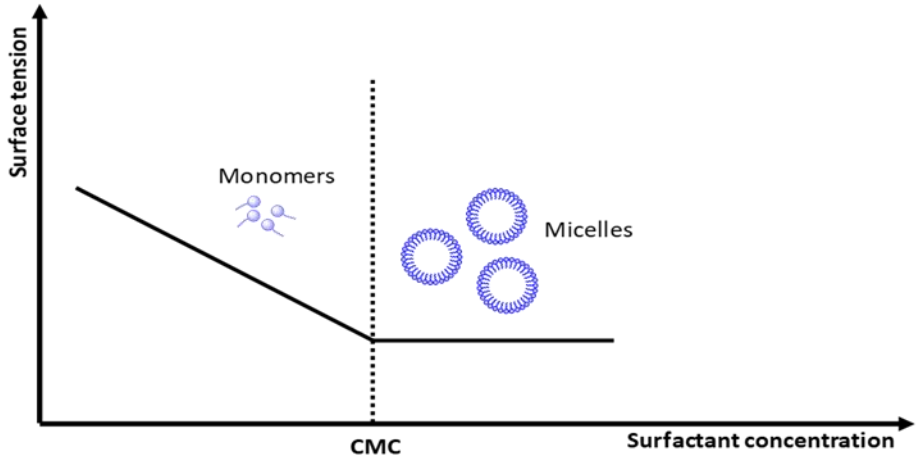


Figure 4. The relationship between surfactant concentration and surface tension enabling the determination of the crtical micelle concentration (CMC).

Table 1. Summary of common experimental techniques that are used to determine micelle structure.[1,2,4,5,6,7,8,9,10,11,12,13,14,15,16]

Micelles characteristics	Techniques								
	TEM	SLS	DLS	SEC	NMR	SANS&SAXS	Ultracentrifugation	Viscometry	Fluorescence
Shape/ size	√								
Molecular weight		√				√	√		
Gyration radius (R_g)		√				√			
R_{core}						√			
Hydrodynamic radius (R_h)			√	√				√	
Chain dynamic					√				√
CMC									√
Hybridization of micelles									√
Micelle density							√		
Dynamic of micelles equilibrium		√							
Unimer weight ratio							√		
Intrinsic viscosity								√	
Macrolattice structures						√			

4. Drug loading and release

In 1984, drug loaded into polymer micelles via chemical conjugation was suggested by the Ringsdorf group.[9.12] In polymeric micelles, loading efficiency is influenced by the hydrophile-hydrophobe balance.[13]

Poorly water-soluble drugs could be encapsulated into the cores of polymeric micelles via three common modes; chemical conjugation, physical entrapment and ionic bonding (e.g. polyionic complexation in the case where the drug and/or polymer display multiple charges).[9]

Dialysis, solid dispersion, oil-in-water emulsions, as well as solvent evaporation are typical methods utilised for loading poorly water-soluble anticancer agents.[6] During chemical conjugation method, the covalent bonds are formed between particular functional groups of the drug and the polymer backbone.[6,13] Physical entrapment of drugs can be achieved by dialysis and emulsion formation.[6] In dialysis, a small quantity of water is added to the solution of drug and polymer followed by dialysis against an excess of water for a sufficient length of time.[6] Generally for poorly water solunle drugs, oil-in-water emulsions are used where the drug is incorporated in the micelle as the oil evaporates/diffuses away.

Release of drugs from the micelles can be affected by several factors, including molecular weight (MW), hydrophilic/hydrophobic balance, solubility of hydrophilic or hydrophobic blocks, and size of micelles.[14]

5. Primary mechanisms of drug release

There are three mechanisms regarded as the major ways to release drugs from the drug-polymer complexes, and these are diffusion, degradation, and swelling.[9,15,16,17]

5.1. Diffusion

By this mechanism the APIs diffuse from the complex through the polymer matrix and the rate of diffusion is controlled by time and distance of diffusion.[16]

5.2. Degradation

Polymers can be designed to degrade under specific conditions, defined by Siepmann and Gopferich as the cleavage of the polymer into oligomers and monomers.[16] During the degradation process the properties of the polymers will undergo to significant changes to the average molecular weight as a function of time (**Figure 5**).[16] If the polymers are in the solid state then the degradation process is either via ”surface degradation” or “bulk degradation”, whereas if the polymers are in the gel/solution state the degradation would typically be a “bulk degradation” process.

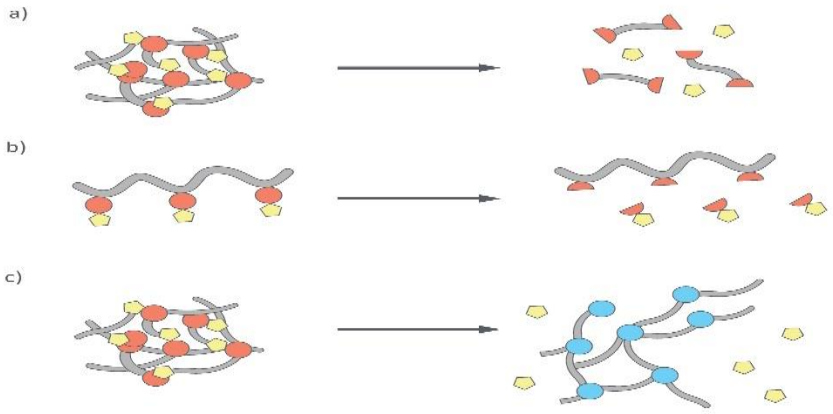


Figure 5. Typical photoactive polymers change triggered by light enabling the release of molecules. A) polymer degradation by irradiation, b) degradation of bottle brush linker to small molecule (e.g. drug), c) change the polarity of polymer encouraging swelling of the polymer matrix and subsequent leaching of the encapsulated substance.

Various stimuli (including heat, light, oxygen, radiation, and enzymes) can induce the degradation of polymers.[9,16] Degradable polymers are popular for DDSs, generally containing labile bonds such as ester, amide, and anhydride bonds that are prone to hydrolysis or enzymatic degradation.[16]

Polyesters are one class of degradable polymers popular for DDSs. Important examples of polyesters used for DDSs are: polylactides (PLA), poly(lactide-co-glycolide) (PLGA), and polycaprolactone.[10,16] Such polyesters degrade by a variety of mechanisms including hydrolysis of the esters (e.g. upon exposure to water or solutions of esterases) and/or exposure to lipases. [18]

5.3. Swelling

The absorption of liquids (e.g. water) can lead to swelling, which leads to growth of the mesh size of polymer due to increase the amount of the solvent within the drug-loaded polymer matrix, which allows diffusion of the drugs/APIs through the polymer matrix to the external environment.[15]

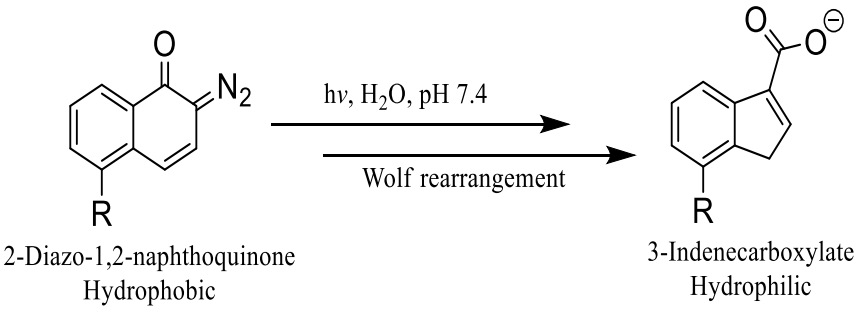
6. Photo- responsive polymeric materials

There are five common mechanisms by which photo-responsive polymer-based materials react including: photoisomerization, photo-induced rearrangement, photocleavage, photo-induced cross-linking, and photo-induced energy conversion (**Table 2**).[19]

Table 2. Illustrate common photoreaction, photo-responsive sets and corresponding of photo-responsive PMs.[19]

Photoreaction	Photo-responsive group	Reaction	Type of Irradiation
Photoisomerization	Azobenzene	Trans to cis or cis to trans	UV
Photo-induced rearrangement	2-Diazo-1,2-naphthoquinone	Wolff rearrangement	UV or NIR
Photocleavage	2-Nitrobenzyl ester	Cleavage	UV or NIR
Photo-induced crosslinking	Coumarin	Dimerization	UV

Among these mechanisms, photo-induced rearrangement is the main mechanism that is emphasised in the first chapter. In biomedical applications, the reaction of photoactive polymers based on photo-induced rearrangement. In this approach, exposure of a photoresist to UV or NIR irradiation causes changes in the solubility of molecules due to changes in the chemical structure (**Scheme 1**).[19] For instance, the hydrophobic 2-diazo-1,2-naphthoquinone-5-sulfonyl chloride (DNQ) is converted to hydrophilic 3-indenecarboxylic acid (3-IC) with a pK_a of 4.5 upon one photo-process (UV) or two-photon process (NIR) via a photo-induced Wolff rearrangement.[19]



Scheme 1. Illustration changing in solubility of DNQ derivatives after photo induced Wolff rearrangement to the hydrophilic 3-IC.

Examples of applications of polymeric micelles in the drug delivery system include: delivery of anticancer agents for therapeutic purposes, delivery of imaging agents for diagnostic purposes, delivery of antifungal agents and polynucleotide therapeutics.[9]

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Chapter 1: Preparation and characterization of photoresponsive hyaluronic acid (HA) micelles loaded with a potential anticancer drug

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Abstract:

Light-sensitive polymeric micelles were prepared to investigate their potential to enhance the delivery of drugs such as doxorubicin (DOX). HA-DNQ bioconjugates may assemble into micelles because the DNQ is hydrophobic and when appended to the backbone of the hydrophilic HA some surfactant-like properties are expected. The irradiation of theHA-DNQ results in a UV-induced Wolff rearrangement reaction converting hydrophobic DNA into the more hydrophilic 3-IC moiety which leads to solubility changes in the HA-DNQ. HA is a natural linear polysaccharide which may enhance the therapeutic effects of drugs *via* improving drug solubility, pharmacokinetics, clearance, and its ability to target cancer stem cells that overexpress cluster of differentiation 44 (CD44). HA-DNQ was characterized by NMR, FTIR and UV-vis. HA-DNQ micelles were prepared and their ability to encapsulate various dyes was studied. Polymeric micelles were characterized by light scattering. The release of dyes from the HA-DNQ micelles was studied.

Key words: Polymeric micelles; light-sensitive polymeric micelles; drug delivery; dialysis; cancer therapy.

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1. Introduction

As controlled drug delivery vehicles, polymeric micelles (PMs), acting as efficient nanocarriers for drugs and pharmaceuticals, offer significant potential in increasing drug delivery to therapeutic sites rather than to healthy cells. PMs are nanocarriers with sizes typically ranging from 10-100 nm by getting spontaneous self-assembly in aqueous media. Recently, PMs have been employed to deliver poorly soluble anticancer agents for different advantages, including small size, bioavailability enhancement, solubilisation of lipophilic drugs and elevated therapeutic index, hence improving drug delivery and accumulation at tumour sites.[1] Consequently, the therapeutic nanoparticles load maybe released according to alterations in the tumour environment, for instance, pH, temperature, specific enzyme or wavelength. Light-sensitive polymeric micelles are among the most promising stimuli-responsive carriers for DDS, e.g. for cancer therapy.[1] Polymeric micelles may be formed from a variety of amphiphilic polymers. Bioconjugation of the hydrophobic block 2-diazo-1,2-napthoquinone (DNQ) to the backbone of the hydrophilic block hyaluronic acid (HA, **Figure 1**) yields micelles.

HA is a naturally occuring linear polysaccharide that consists of repeating disaccharide unit of N-acetyl-D-glucosamine and β-glucoronic acid,[2,3] which is hydrophilic (pKa of carboxyl group ranges between 3 and 4). HA-based hydrogels and bioconjugates have been developed as DDS. The HA leads to enhanced therapeutic effects *via* improving drug solubility, pharmacokinetics, clearance, and its ability to target cancer stem cells that overexpress the cell surface glycoprotein Cluster of Differentiation 44 (CD44),[3,4,5,6] which is an important hyaluronan receptor which plays a role in turnover regulation. [3.4.5.6] Hyaluronic acid anchors to the cells through CD44 which yields a hydrated environment in the extracellular matrix, thereby ensuring nutrient supplyand visco-elastic properties.[4,5,6]

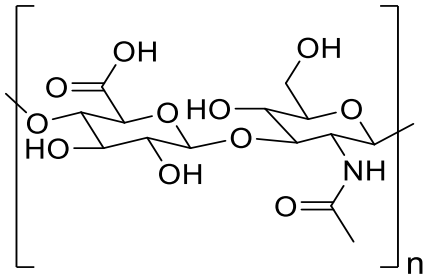


Figure 1. Chemical structure of HA.

2-diazo-1,2-napthoquinone (DNQ, **Figure 2**) is a conventional photoactive compound (PAC) that is used as a positive photoresist.[7] It is able to change molecular structure (**Scheme 1&2**) which leads to alteration of the molecule's solubility through a UV-induced Wolff rearrangement reaction.[7]

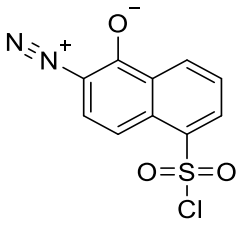
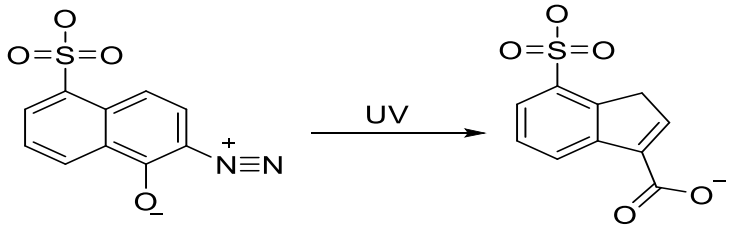
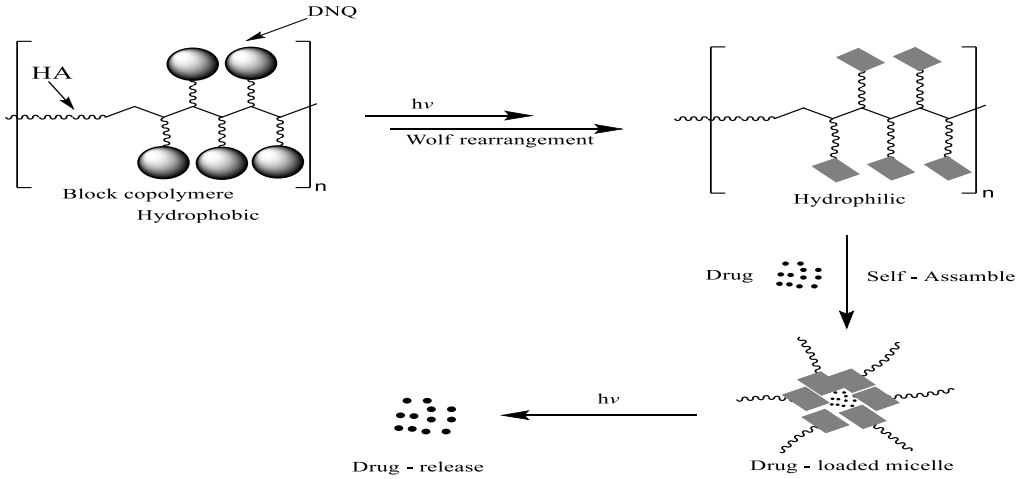


Figure 2. Chemical structure of DNQ.



Scheme 1. Shows the change in the chemical structure of DNQ which leads to alterationof the molecule's solubility through a UV-induced Wolff rearrangement reaction.



Scheme 2. Shows the self-assembly of HA-DMQ and photo-induced Wolff rearrangement, which dissociated the structure of micelles upon UV irradiation to enhance extracellular release HA.

2. Experimental section

2.1. Materials

All materials were of analytical grade or higher and were used as supplied. Sodium hyaluronate (Mw = 1.5 – 2.2 million Da) was obtained from Alfa Aesar (Lancashire, UK). 2-Diazo-1,2-naphthoquinone-5-sulfonyl chloride (sc-DNQ, 95%) was purchased by Key Organics (UK) and was stored in the dark. Trimethylamine hydrochloride 98% was purchased by Acros Organic. Nile red (99%) was supplied from Acros Organic. Coumarin 102, methylene blue, high purity, biological stain and eosin b were purchased from Alfa Aesar. Triethylamine, N,N-dimethylformamide (anhydrous 98%) and dialysis tubing (Fisherbrand^{TM} Regenerated Cellulose, MWCO 3500 Da) were purchased from Fisher Scientific (Loughborough, UK).

2.2. Methods

2.2.1. Synthesis of HA-DNQ amphiphilic copolymer

The synthesis of HA-DNQ by conjugating 2-diazo-1,2-napthoquinone (DNQ) to the backbone of the HA was reported by Vasileios Oilkonomou in the Hardy lab in 2018. The HA-DNQ was re-synthesised over the course of this MSc thesis to make study it via a variety of experiments. The hydrophilic hyaluronic acid, was modified with hydrophobic sc-DNQ by adaptation of the literature.[8] Briefly, hyaluronic acid 500 mg (0.04 mmol) was dissolved in 75 mL of deionised (DI) water and the mixture was stirred for 24 h, then triethylamine (0.5 mL, 4.9 mmol) and trimethylamine hydrochloride (0.02 g, 0.34 mmol) were added. 1.0 g (3.72 mmol) of light-responsive sc-DNQ was diluted in 7.5 mL of DMF at room temperature and added dropwise to the hyaluronic acid solution. The mixture was stirred at room temperature in the dark (wrapped in aluminum foil) for 48 hours. The product was purified by precipitation in acetone, followed by washing the product in cold methanol (repeated 4 times) and Soxhlet extraction with acetone to remove any traces of low molecular weight contaminants

(e.g. sc-DNQ). Finally, the product was dried under vacuum at room temperature, yielding an orange solid (100.51 mg).

2.2.2. Preparation of AH-DNQ micelle

Dialysis was utilized to prepare HA-DNQ micelles. Briefly, 3 mg of HA-DNQ was dissolved in DMSO (1 mL), then 1 mL of distilled water was added dropwise. The mixture was stirred roughly for 6 h, the solution was dialyzed against deionized water in a dialysis membrane (MWCO 3500 Da) for 3 days to remove the DMSO.

2.2.3. Loading of dyes (Coumarin 102, Methylene Blue and Eosin B)

For the preparation of micelles encapsulating dyes (coumarin 102, methylene blue and eosin b), 3 mg of HA-DNQ and 0.06 mg of dye were dissolved in 1 mL PBS. Then, 1 mL of DI water dropwise was added into the solution and stirred roughly for 6 h. The mixture was dialyzed against deionized water (Fisherbrand^{TM} regenerated Cellulose, MWCO 3500 Da) for 3 days to obtain dye loaded HA-DNQ micelles. The same procedure was repeated by replacing PBS with DMSO in the initial dissolution step.

2.2.4. Critical micelle concentration (CMC)

Nile Red was used as a fluorescent probe to determine the CMC of HA-DNQ. Fluorescence spectra were obtained at room temperature utilizing an Agilent Eclipse Fluorescence Spectrophotometer. Fluorescent probe encapsulated micelles were selected and diluted for the measurement of CMC. Fluorescence spectra were recorded at excitation wavelength from up 550 nm to 720 nm. The concentration of the HA-DNQ micelles was varied 0.00001 mg/mL to 0.06 mg/mL.

2.2.5. Light irradiation setup

At various time points, light irradiation was achieved by using a ThorLabs light emitting diodes (LED) DC4104 Driver system with a LED (365) nm in a light proof container (outdoor plastic garden shed manufactured by Keter).

2.6. Characterizations

2.6.1. Fourier transform infrared (FTIR)

FTIR spectroscopy was applied to provide qualitative molecule identification. It was recorded on a Cary 630 FTIR (Agilent Technology) spectrophotometer at room temperature in the wavelength ranging from 500 to 4000 cm^{-1}.

2.6.2. Nuclear Magnetic Resonance

^{1}H NMR spectra of the HA-DNQ was recorded in D_2O using a Bruker 500 MHz Ultrashield plus NMR spectrometer. Singlet (s), doublet (d), multiplet (m) were used as notation for ^{1}H NMR spectral splitting patterns. The NMR experiments were recorded in the range of 0–12 ppm, the concentration of the polymers was 3 $mg.mL^{-1}$.

2.6.3. UV-vis spectra

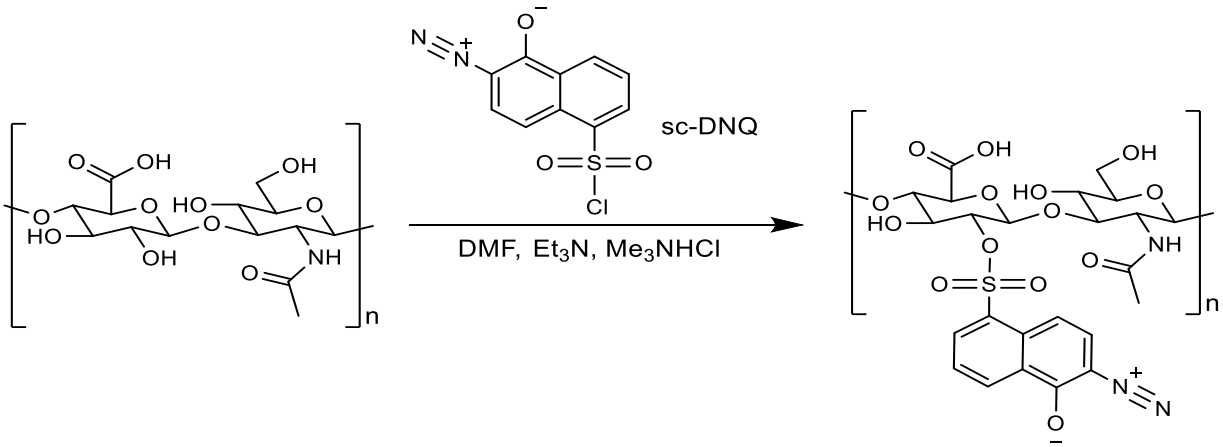
UV-vis spectra of sc-DNQ with different concentrations and the polymers after irradiation for different time were carried out with a UV-vis Thermo scientific™ NanoDrop Spectrophotometer. Spectra were collected within a range of 300–600 nm.

2.6.4. Light-Emitting Diode (LED)

Light irradiation was achieved by using a ThorLabs light emitting diode (LED) DC4104 Driver system with a LED (365) nm in light proof container (outdoor plastic garden shed manufactured by Keter).

3. Results and discussion

The conjugates of HA-DNQ were prepared by the modification of hydrophilic HA with hydrophobic sc-DNQ (**Scheme 3**). The ratio of HA to DNQ is presented in table 1.

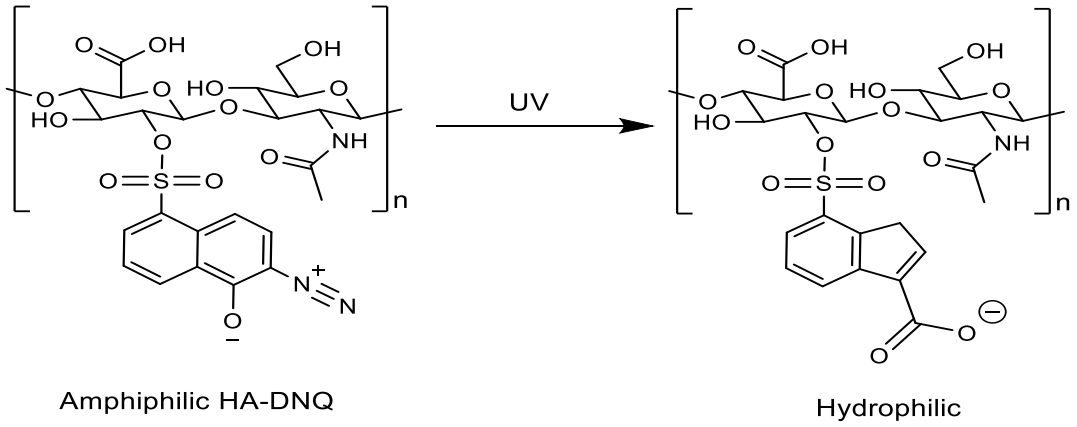


Scheme 3. Synthesis route of HA-DNQ.

Table 1. Formulation of HA-DNQ

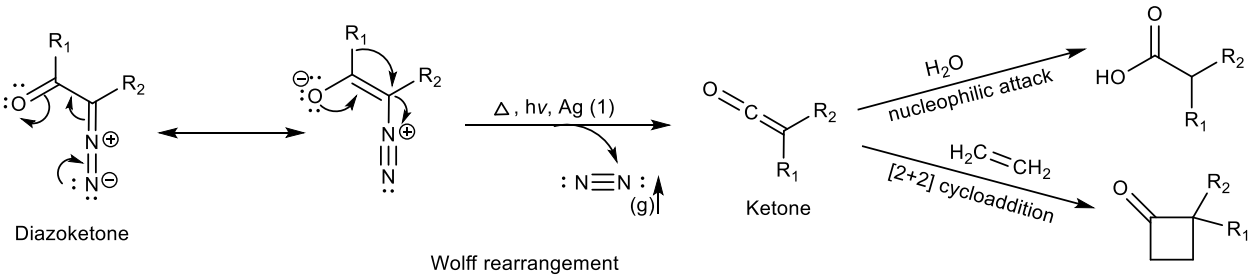
Polymer	Weight ratio of HA-DNQ
HA-DNQ	0.5 : 1

The use of photo-induced rearrangement of polymeric micelles has potential for biomedical applications due to the ability to load and release insoluble/poorly soluble drugs in their interior. Indeed, upon exposure to specific wavelengths of light, the hydrophobic (DNQ) was converted into hydrophilic moiety (3-indenecarboxylic acid (3-IC)) through a UV-induced Wolff rearrangement reaction which led to solubility changes in the HA-DNQ bioconjugates (**Scheme 4**).



Scheme 4. Conversion of amphiphilic HA-DNQ to hydrophilic HA-3-IC.

The chemical bond that provides a nitrogen group (e.g. the diazo) is sensitive to light. In 1944, Süs referred to that indenecarboxylic acid is the primary product produced by the irradiation of 2-diazo-1,2-naphthoquinone in aqueous acidic solution. He also suggested a mechanism for this reaction via Wolff rearrangement (for the ring contraction) through ketocarbene (**Scheme 5**). When DNQ and its derivatives are exposed to UV or NIR irradiation, the molecule will be excited and then the reaction will take place in water followed by releasing N_2 to produce a carboxylic acid. DNQ is an organic compound that is soluble in most organic solvents (but not in water) used as a positive photoresist, and the product of the photoconversation of DNQ is 3-indenecarboxylic acid (3-IC) which is polar and soluble in polar solvents including water.



Scheme 5. General mechanism of the Wolff rearrangement.

3 mg of the final product of HA-DNQ was diluted in approximately 1 ml of d6-DMSO, then 5 µl of that solution was transferred to an NMR tube with 500 µl D_2O. ^1H NMR spectroscopic analysis of the modified HA-DNQ shown in **figure 3** indicated the successful synthesis of HA-DNQ. The poor signal to noise ratio is caused by the very low concentration of the HA-DNQ in water and will have to be recorded again prior to contemplation of publications.

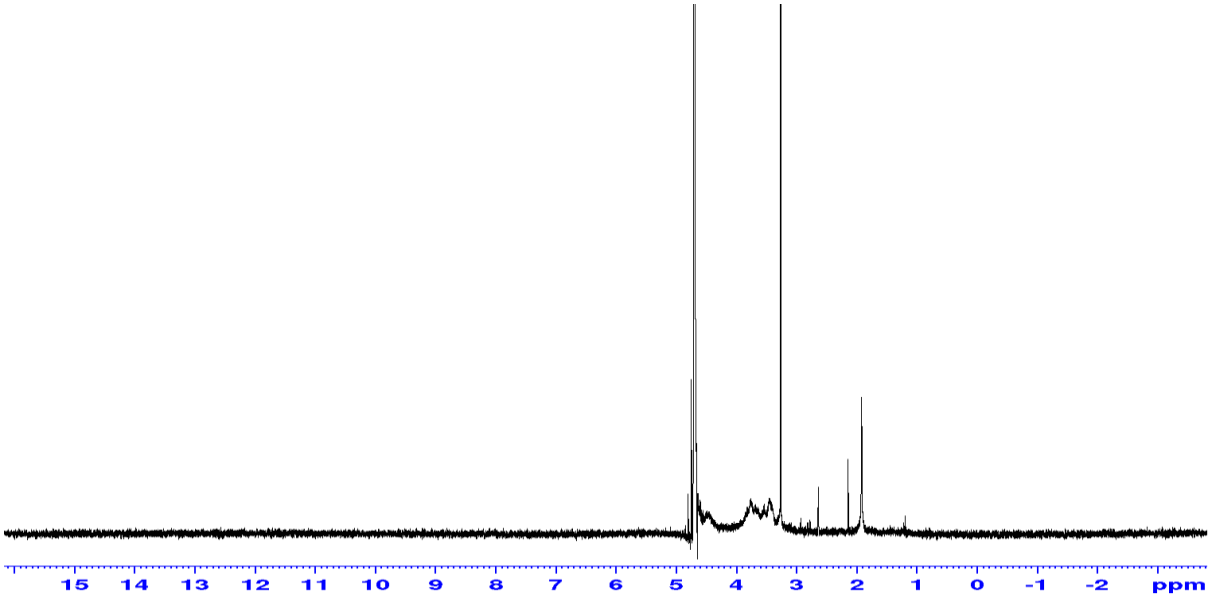


Figure 3. ^{1}H NMR spectrum of HA-DNQ.

The FTIR spectra of HA, DNQ and HA-DNQ were recorded. The FTIR spectrum of DNQ is shown in **Figure 4**, absorption peak of C=C, C-O and C-N bonds are observed at 1558 cm^{-1}, 1366 cm^{-1} and 1166 cm^{-1}, respectively. The FTIR spectrum of HA is shown in **Figure 5**, enabling observation of hydroxyl groups at 3443 cm^{-1}, bands at 1648 cm^{-1} and 1720 cm^{-1} corresponded to carbonyl stretching bonds of amide and carboxylic acid, respectively. The FTIR spectrum for HA-DNQ shown in **Figure 6** confirms the successful conjugation of DNQ onto the backbone of HA with the characteristic (albeit weak) absorption peak of the diazo in the DNQ at ca. 2200 cm^{-1}, the peak at 1723 cm^{-1} was observed in the spectrum of HA-DNQ which also confirms the formation of sulfonate ester formation. The relatively low level of DNQ conjugation is not ideal, but still enough to impart some amphiphilicity to the HA-DNQ bioconjugate.

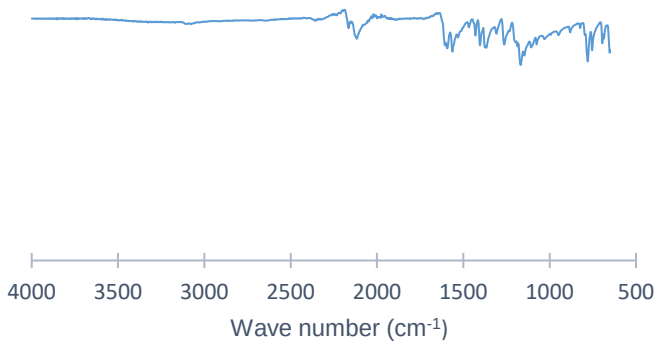


Figure 4. FTIR of DNQ.

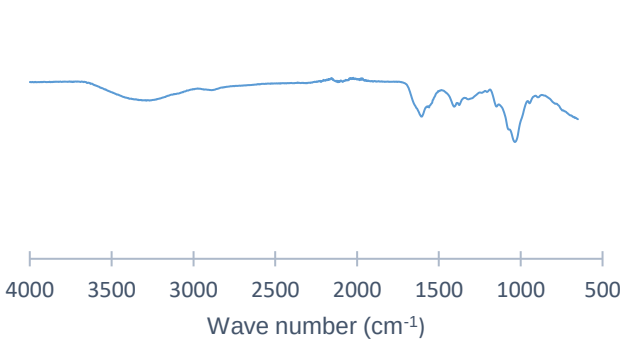


Figure 5. FTIR of HA.

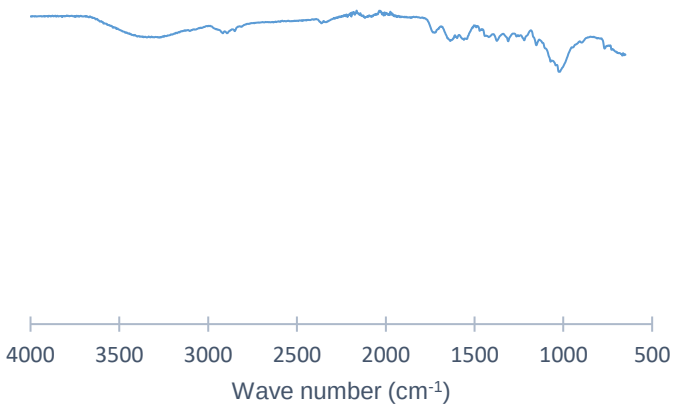


Figure 6. FTIR of HA-DNQ.

UV-vis spectra also provided evidence for the successful conjugation. For UV-vis, HA-DNQ was measured in DMSO and PBS, respectively. The absorption peak of DNQ appeared at 403 nm (**Figures 7 & 8**).

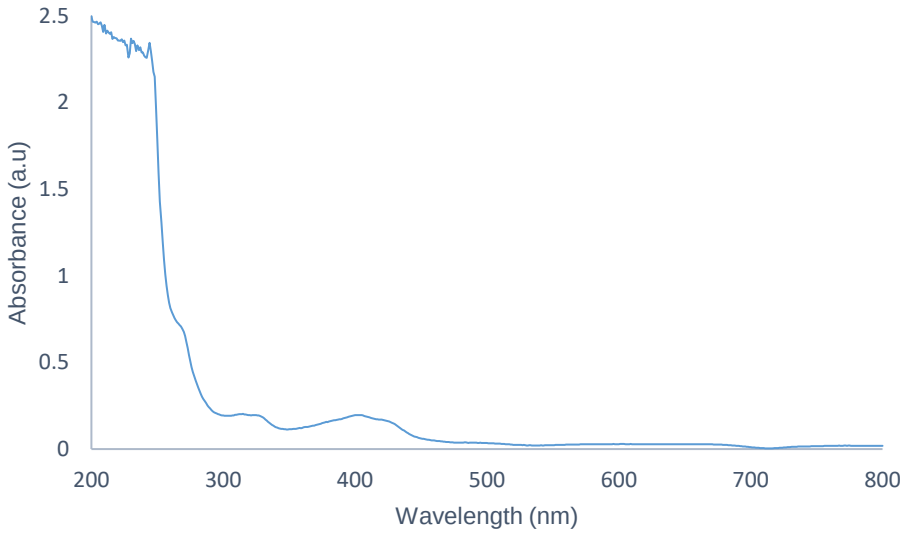


Figure 7. UV-vis spectra of HA-DNQ (3 mg/ml in DMSO).

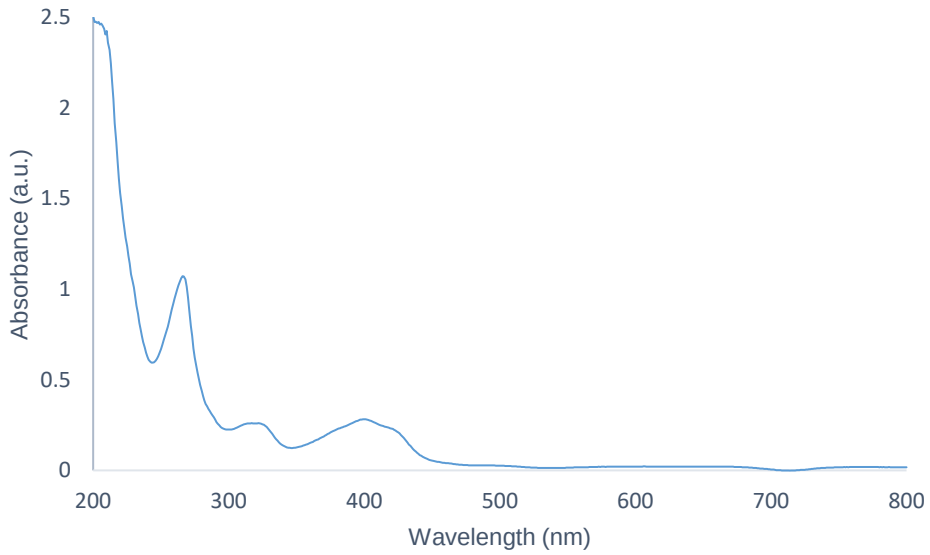


Figure 8. UV-vis spectra of HA-DNQ (3 mg/ml in PBS).

The light-sensitivity of the HA-DNQ was demonstrated by changes in UV-vis spectra in PBS solutions at various times (**Figure 9**). The characteristic absorption peak of DNQ group at 403 nm steadily decreased with increasing duration of irradiation (up to 5 minutes is shown in **figure 9**.

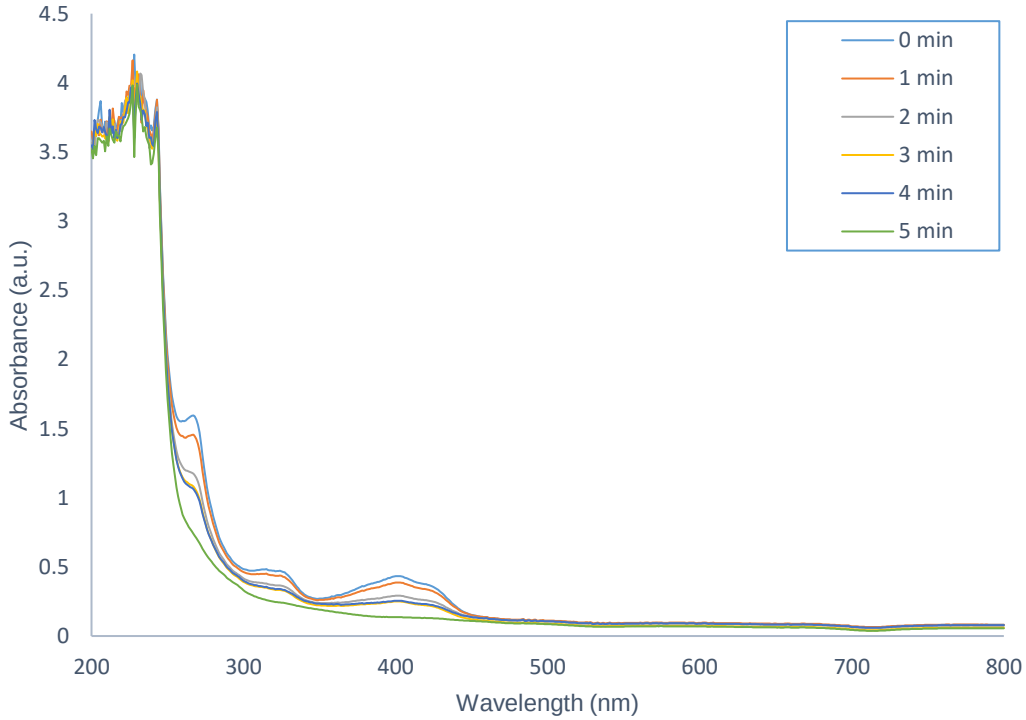


Figure 9. UV-vis spectra of HA-DNQ (3 mg/ml in PBS) after dialysis and irradiation with 365 nm light for various times (ET= elapsed time).

A dialysis method was used to prepare HA-DNQ micelles and subsequently study their critical micelle concentrations (CMC) utilising Nile Red as a fluorescence probe (**Figure 10**). The fluorescence emission spectra of Nile Red (λ_{exc} 550 nm) in HA-DNQ micelles of varying concentrations highlights differences in fluorescence and therefore the hydrophobic microenvironments in the micelles.

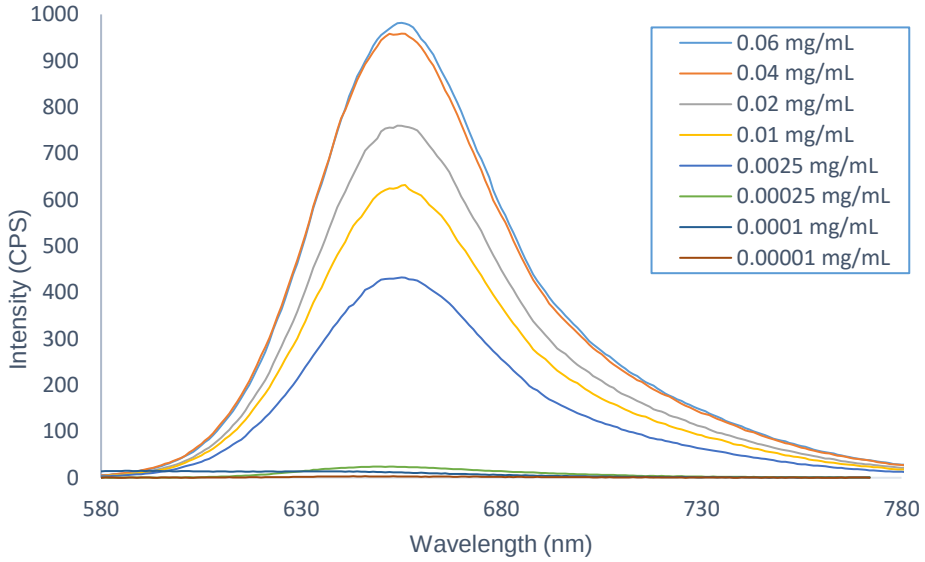


Figure 10. Fluorescence emission spectra of Nile Red in HA-DNQ micelles of varying concentrations.

Plotting the fluorescence intensity vs. log of concentration of HA-DNQ (**Figure 11**) enabled the estimation of the CMC of HA-DNQ to be ca. 0.001 mg/ml.

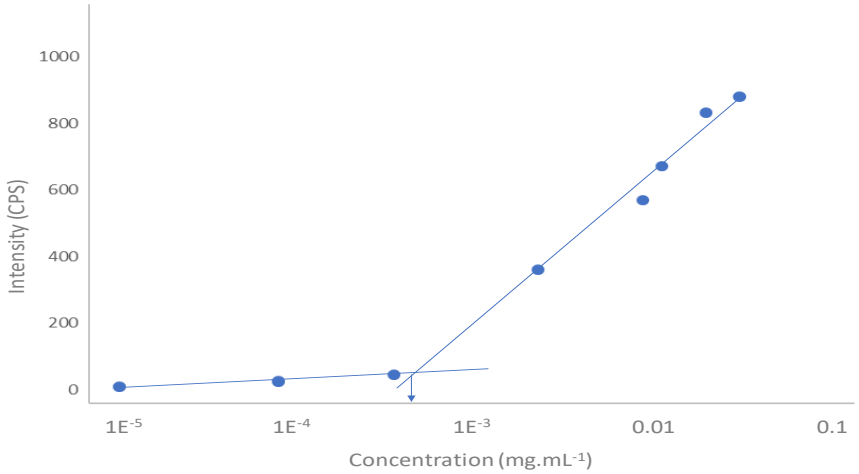


Figure 11. Plot of the emission intensity versus the log of concentrations of HA-DNQ micelles.

The controlled release of dyes from the HN-DNQ micelles after irradiation with UV light (365 nm) at different times was studied with the drug diffusing through the dialysis membrane. As a first experiment changes in UV spectra of the polymer with exposure to light was measured (**Figure 12**), in which we observed photogradation.

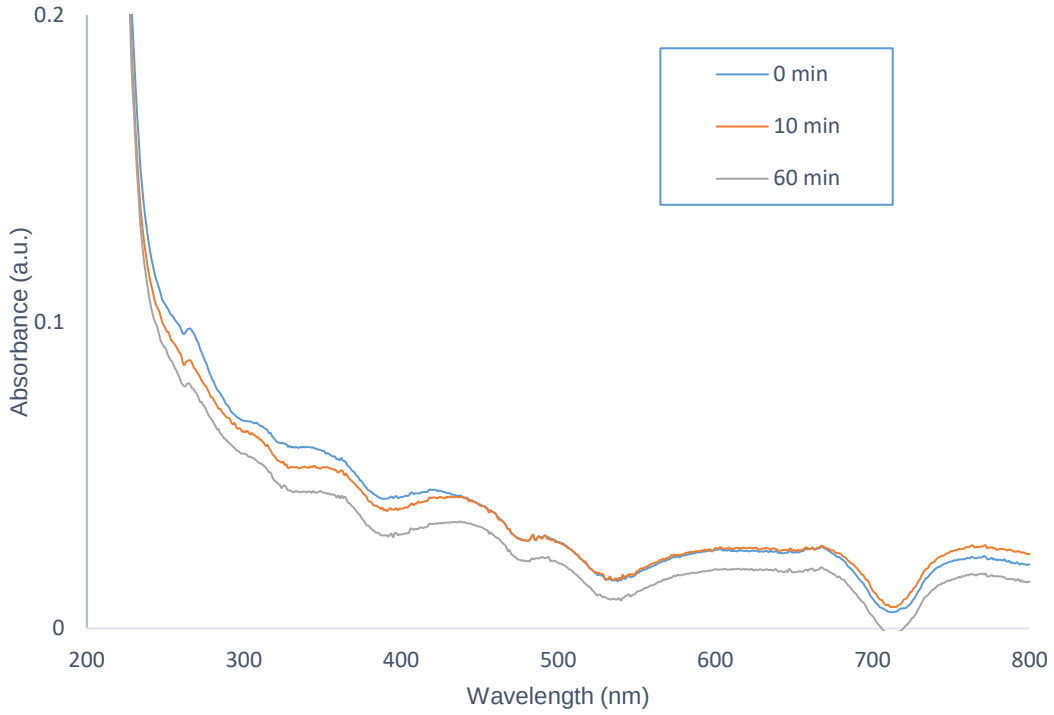


Figure 12. UV-vis spectra of HA-DNQ after dialysis and irradiation with 365 nm light for various times (ET= elapsed time).

Several dyes were selected as model hydrophobic drugs to investigate the photo-triggered and photo-controlled release behaviour of HA-DNQ in PBS and DMSO, respectively, examples with Coumarin 102 are depicted in (**Figures 13 &14, Figure A1**).

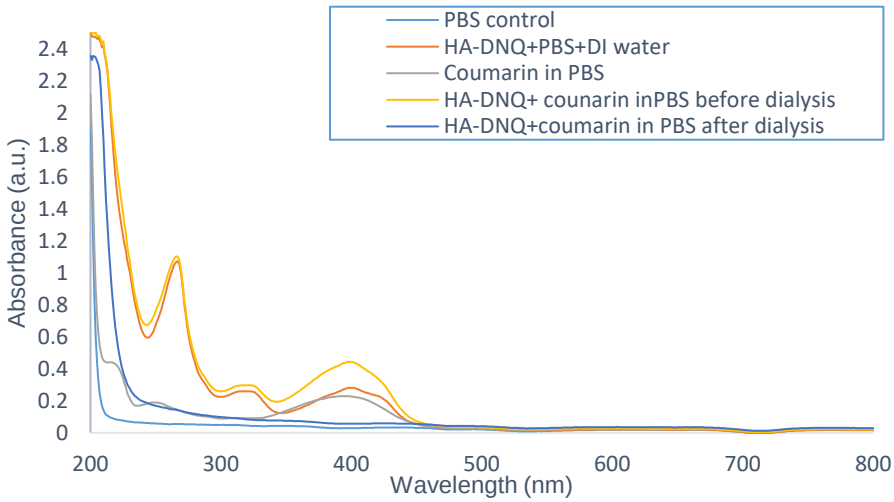


Figure 13. UV-vis spectra of HA-DNQ micelle loaded with coumarin 102.

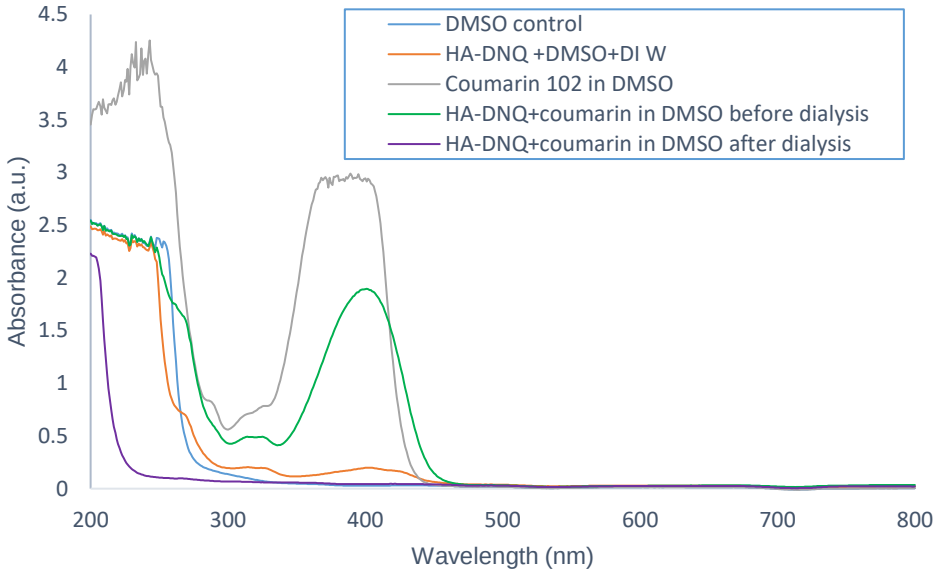


Figure 14. UV-vis spectra of HA-DNQ micelle loaded with coumarin 102.

The characteristic peak of coumarin 102 (at λ_{max} 390 nm) is observed to markedly diminish after dialysis due to the release of dyes by diffusion during the dialysis process. In order to further confirm the process of drug release; we did experiment with fluorescence spectra (**Figure 15**).

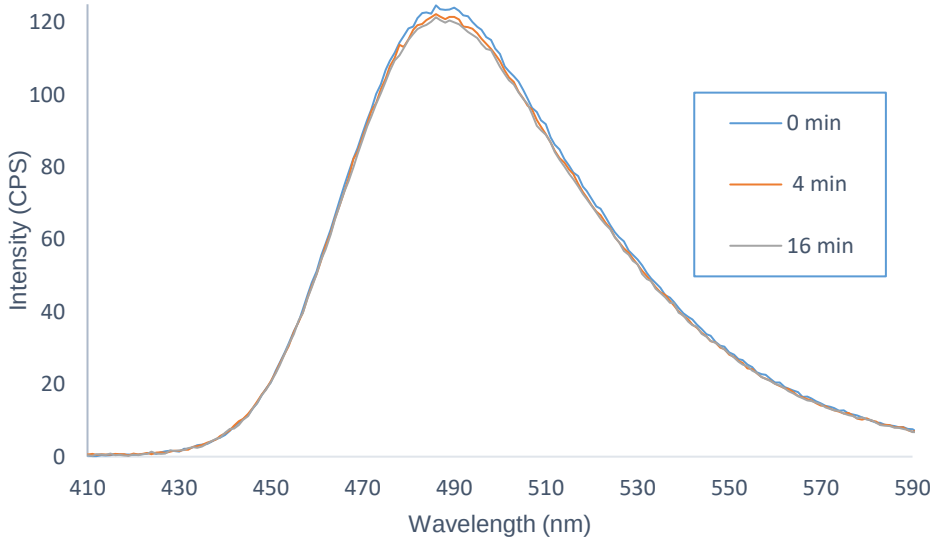


Figure 15. Fluorescence emission spectra of HA-DNQ micelles loaded coumarin 102 at various times (λ_{exc} = 420 nm).

The fluorescence of dyes may be increased substantially in the hydrophobic core of micelles. The release of coumarin 102 was observed after irradiation with UV light at 365 nm. We observed that the maximum fluorescence was at 0 minand after the light was turned on, the value of absorption peak decreased moderately.

Similar leaching of the drug during the dialysis process was observed spectroscopically for methylene blue **(Figure A2 & A4)** and eosin B **(Figure A3 & A5);**

confirmed via photography in **figure A6**. Clearly more detailed studies are necessary to optimise loading of the drugs, ascertain the maximum loading level and study the controllable release of a drug that stays in the micelles after the dialysis process.

4. Conclusion

Having successfully prepared and attempted to encapsulate of HA-DNQ micelles with several dyes, we tested their responses to irradiation with UV light (365 nm) to understand their potential for application as drug carriers. Irradiation with light led to increasing hydrophilicity and dissociation of the structure of micelles via the Wolff rearrangement of DNQ to 3-IC demonstrating their potential for applicaton as drug carriers. Clearly a multitude of further experiments must be undertaken to study the micelles properties in further detail, to optimise the drug loading process and assess a wide variety of drugs for their potential for encapsulation and release.

5. Reference

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Appendix A:

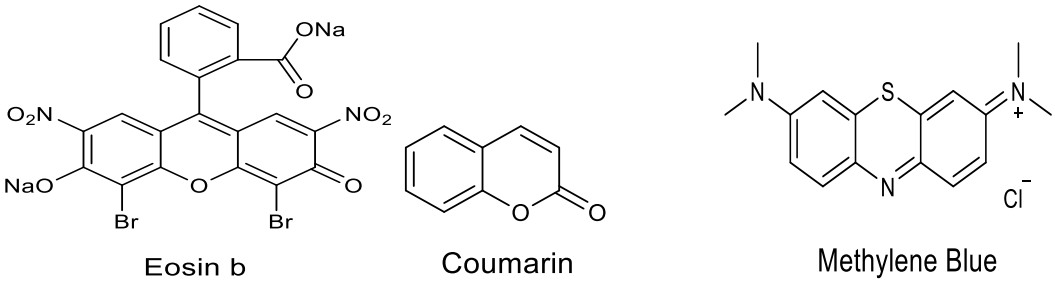

Figure A1. Shows the chemical structures of eosin b, methylene blue and coumarin 102.

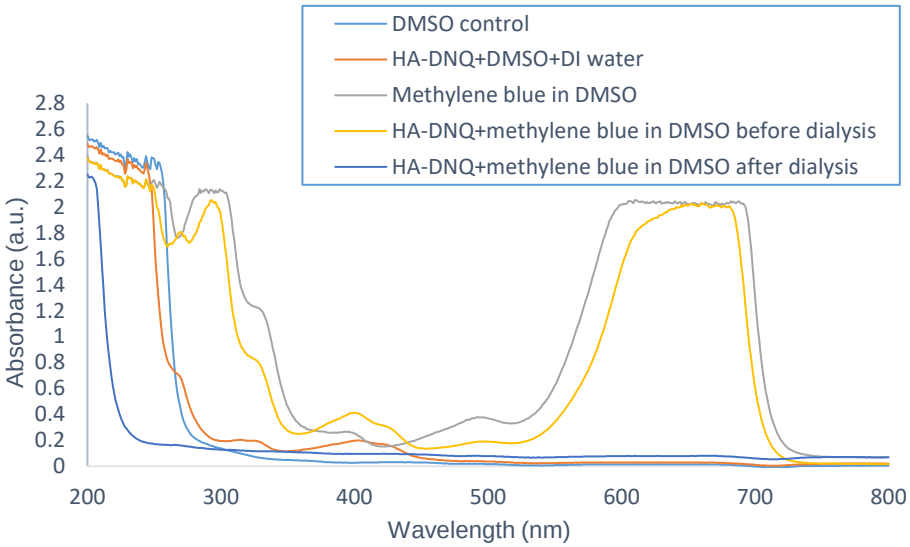

Figure A2. UV-vis spectra of HA-DNQ micelle loading methylene blue.

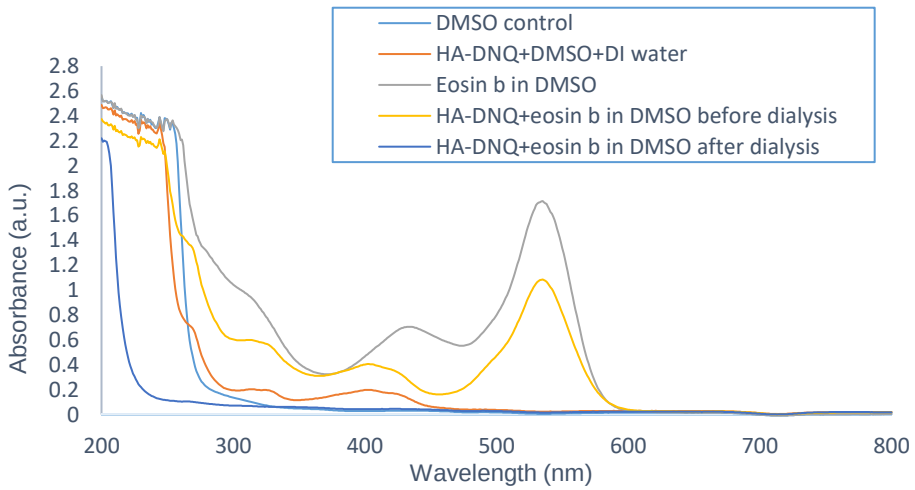

Figure A3. UV-vis spectra of HA-DNQ micelle loading of eosin b.

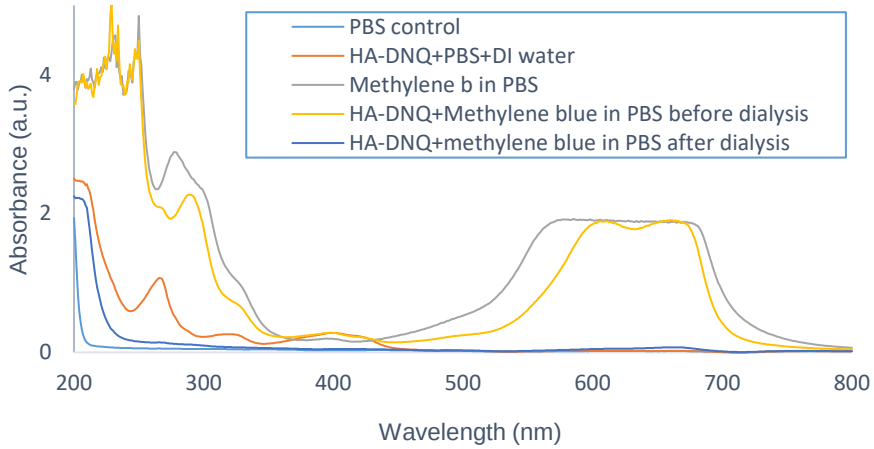

Figure A4. UV-vis spectra of HA-DNQ micelle loading of methylene blue.

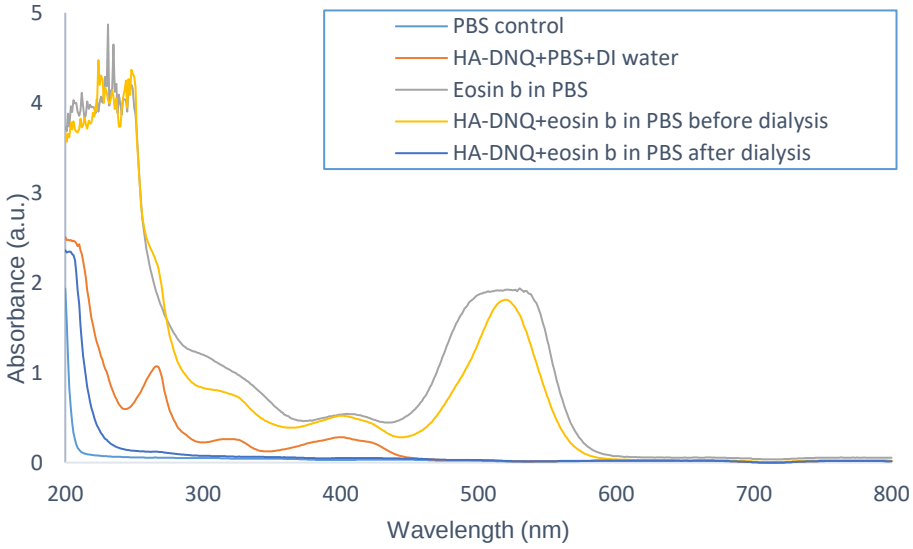

Figure A5. UV-vis spectra of HA-DNQ micelle loading of eosin b.

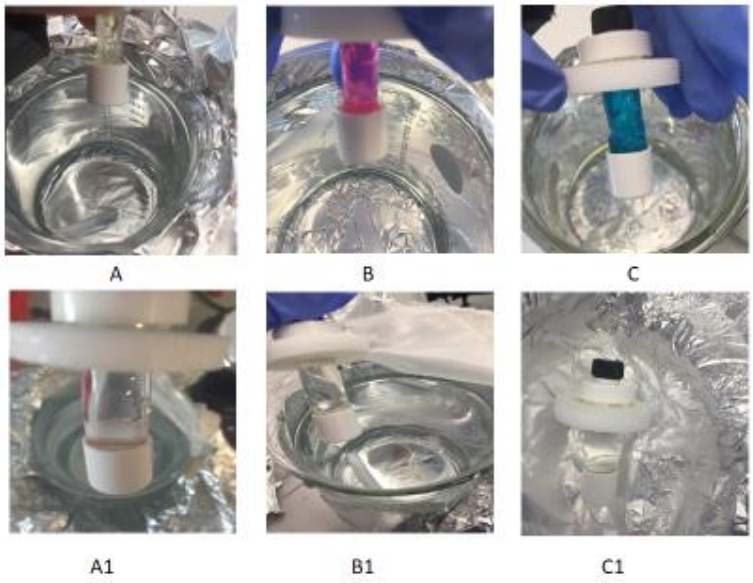

Figure A6. A,B,C shows the differences in solution appearances of coumarin 102, eosin b and methylene Blue**,** respectively**,** before dialysis for 3 days, while figures of A1,B1 and C1 and after dialysis.

Chapter 2. Synthesis of photoactive hydrogels for light-triggered drug delivery

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Abstract:

Poly ethylene (glycol) diacrylate (PEGDA) based hydrogels have been prepared from commercially available chemical synthesised by differing concentration of non-photolabile crosslinkers and photolabile crosslinkers. The influence of varying crosslinking agent in network swelling behaviour and in order to improve the hydrophilicity of the backbone and achieve better drug release results has been tested. The photoactive hydrogels were prepared by conjugation of 2-nitrobenzyl groups to PEG derivatives.

Keywords: Hydrogels; polymeric gels; cross-linking agents; photocleavable; drug delivery; swelling.

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1. Introduction:

Hydrogels are a three-dimensional network that constitutes a group of polymeric substances having hydrophilic properties. They are capable of retaining a large amount of water or biological fluids in their swollen state.[1]

In 1960, the first soft contact lens was prepared by hydrogel based on poly-2-hydroxyethylmethacrylate (PHEMA) with typical properties (figure 1). Over the past few decades, engineering of hydrogels has resulted in the development of simple networks and smart hydrogels.[2] Hydrogel engineering includes development of a broad range of monomers/polymers/initiators by chemical modification, which has enabled the development of smart hydrogels that are able to respond to specific physical or chemical stimuli (such as temperature, pH and light) or be 3D printed.[2,3]

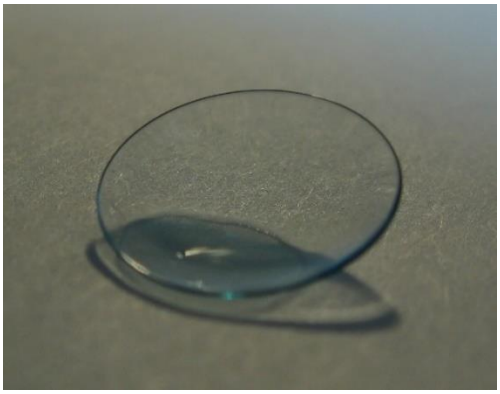


Figure 1. Soft contact lens contains N,N-dimethylacrylamide based hydrogel (from Wikimedia Commons: https://commons.wikimedia.org/wiki/File:Contact_lens_1.jpg).

There are two major categories of hydrogels based upon the building blocks used to prepare them (i.e. natural and synthetic). The natural hydrogels are represented by hyaluronic acid, fibrin, collagen, chitosan, silk and derivatives thereof. Whereas, gels composed of synthetic polymers poly(ethylene glycol), poly(ethylene glycol)acrylate and poly(vinyl alcohol) form synthetic gels.[4]

There are several classical chemical methods that can be utilised to synthesise hydrogels. This could involve one step-procedures prior to polymerisation or multiple step-procedures (e.g. to produce polymers that have reactive sites and their subsequent cross-linking or lead to react such polymers with appropriate cross-linking agents).[4]

The network is the main structure of a hydrogel, which is either physically entangled or chemically formed *via* non-covalent or covalent interactions, respectively. The degree of crosslinking possesses a significant influence on the hydrogel structure and its swelling capacity. The greater the extent of crosslinking, the lower the flexibility of a hydrogel to swell, shrink, or respond to different stimuli.[4]

2. Hydrogel properties

Consequently, the general aim of engineering and design of hydrogels is to produce materials with mechanical properties suited to the specific application, controlled swelling and simple synthesis, potentially with the ability to respond to physical, chemical and biological stimuli.[4]

2.1. Swelling

The absorption of water or other liquids may lead to swelling, which leads to the growth of the mesh size of the polymer due to increasing the amount of the solvent within the drug-loaded polymer matrix, which allows the diffusion of the drugs/APIs through the polymer matrix to the external environment.[5,6.7] Hydrogels can potentially absorb from around 10-20% up to thousands of times their dry weight in water.[8]

The process of swelling begins when the dry hydrogel absorbs water, then molecules of water entering the matrix will hydrate hydrophilic groups and lead to form primary bound water. When the polar moieties are hydrated, the network swells and potentially exposes hydrophobic molecules, which also interact with water molecules, leading to

secondary bound water.[6] The total bound water is the combination of both primary and secondary bound water molecules. As both hydrophobic and hydrophilic groups have bound water molecules; more water will be absorbed due to osmotic driving force of the network chains called free or bulk water. It is anticipated that this bulk water fills the space between the network chains, and/or the center of larger pores, macropores or voids. As a result the additional swelling of hydrogel occur.[8] At a certain limit, the chemical and physical crosslinks will prevent additional swelling resulting in an elastic retraction force as a result the hydrogel reaches an equilibrium swelling level.[8] After swelling, the gel may collapse/dissolve depending upon the composition and degradable properties of polymer chains.[8]

Estimating the relative amounts of free and bound water can be done by several methods, which emphasised by different researchers, such as fractions of the total water content, differential scanning calorimetry analysis (DSC) and nuclear magnetic resonance spectroscopy (NMR).[8] Measuring the weight of dry and the swollen-state gels can be used to evaluate and calculate either the ponderal variation (water uptake) or the volume of adsorbed solvent (both quantities are considered as percentages).[1,8]

The valuation of swelling and stability state of swelling is one of the most accurate, simplest, and inexpensive methods. It is suitable for determining many hydrogels characteristics such as the degree of crosslinking, the rate of degradation and other mechanical properties. It is considered an appropriate means to discriminate between crosslinked gels and the non-crosslinked original polymer.[8]

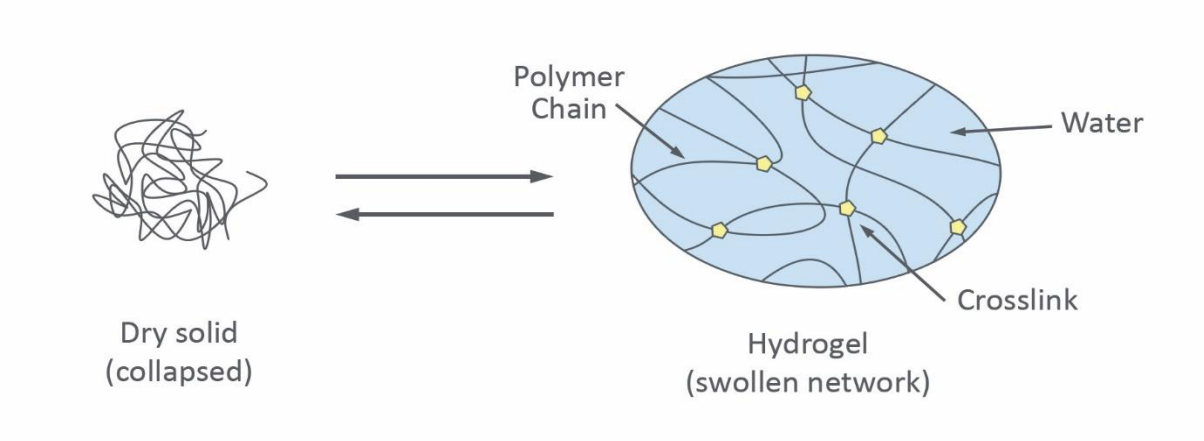


Figure 2. Illustration of the network structure of dry hydrogel and swelling after immersion into a solvent (Adapted from [9]).

2.2. Crosslinking

The degree of crosslinking is the main characteristic of a hydrogel. The process of hydrogel's matrix formation can be categorised into two main classes: the first class is the physical crosslinking (e.g. hydrophobic interactions between chains and ionic interactions between a polyanion and a polycation or between a polyanion and multivalent cations).[8] The second class is chemical crosslinking (e.g. Michael's reaction or nucleophile addition), potentially formed by UV-light or heating. Properties of the hydrogel can be controlled by tuning the degree of crosslinking. [8]

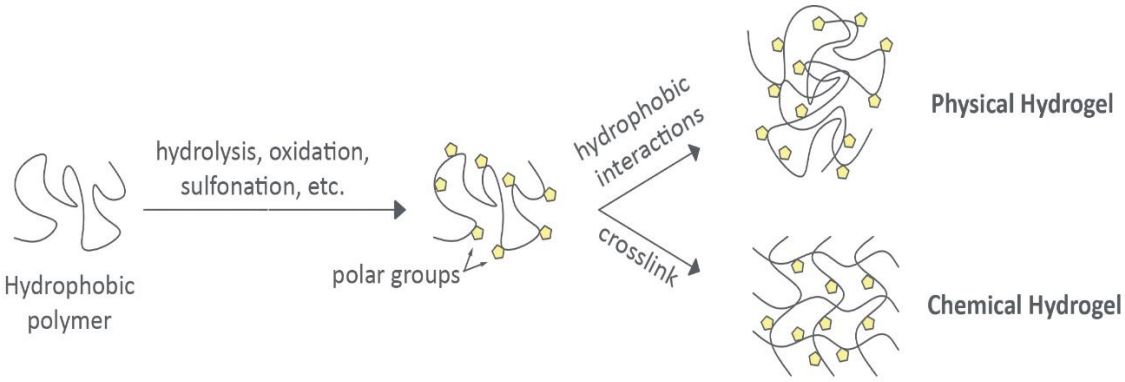


Figure 3. Illustration hydrogel's matrix formation based on gels categories (Adapted from [3]).

2.3. Mechanical properties

The mechanical properties determine the potential applications of thehydrogels and can be changed according to a multitude of variables.[8]

2.4. Porosity and permeation

Pores in hydrogels can be formed in the interstitial space between chemical crosslinks or by phase separation of hydrophobic portions of the polymers constituting the sample spanning network or indeed sacrificial porogens, which underpin the average pore size, the size distribution of pore and the pore interconnections.[8] Three main factors can affect pore-size distributions of hydrogels, including the chemical crosslinks concentration that is determined by the ratio of initial crosslinker to monomer/macromonomer.[8] In this case, the physical entanglements concentration of the polymer strands can be determined by the initial concentration of all polymerisable monomers in the aqueous media.[8] The initial concentration of the cationic or anionic monomer offers a means of controlling the net charge of the network of a polyelectrolyte hydrogel.[8]

The structure of the pores of the hydrogel can also be affected by the properties of the surrounding environments such as solution, particularly by dissolved ionic solutes and dissolved uncharged solutes which partition unevenly between the gel phase and the solution phase (Osmotic effects).[8] Techniques which are employed to investigate the porosity of hydrogels include microscopy (scanning electron, microtomography, liquid displacement, etc.) and theoretical methods.[8]

Stimuli-sensitive hydrogels can undergo specific changes in the swelling ratio upon application of a stimulus (e.g. pH, light, temperature and electric field). Among them, light-sensitive hydrogels have been investigated for the development of drug delivery systems based on polymers with photoactive groups in the hydrogel.[10]

3. Light-sensitive hydrogels

Light-sensitive hydrogels can respond to a variety of wavelengths of light dependent on their specific chemical structures.[11] Chromophores which are able to absorb and then emit light, often lead to increases in the temperature of the hydrogels.[7] These light-sensitive gels function through three different mechanisms. The first mechanism generates heat by converting light to heat through specific light-sensitive moieties contained in heat-sensitive materials. When the gel generates heat and increases temperature to gel phase transition temperature, hydrogel-sensitive to this temperature alteration leads to the gelation of the system.[7] The second mechanism depends on the ionisation of photo-sensitive molecules, which produce a large number of ions upon exposure to light.[7] A resulting sharp ion concentration gradient, inside and outside the gel, leads to an osmotic pressure difference and eventually, the gel expansion. The third mechanism is based on combining chromophoric groups into the matrix of the hydrogel. As a result, physiochemical properties (e.g. geometry and dipole moment) will change the ability to respond to light.[7]

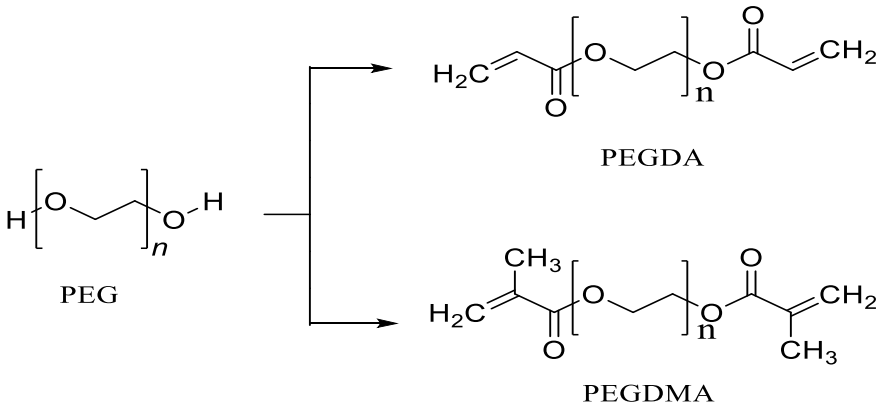
UV-sensitive hydrogels were prepared by introducing a leuco-derivative molecule (e.g. bis(4-dimethylamino)phenylmethyl leucocyanide), into the structure of polymer due to the ability of such leuco derivatives to ionise upon UV irradiation.[11] At a stable temperature and responding to UV light, the hydrogels will be swelled, but when removing UV irradiation, the hydrogels will shrink. Upon application of the UV light, swelling can be induced *via* an increase in osmotic pressure inside the hydrogel due to the formation of cyanide ions by UV light (clearly highly toxic and therefore of no medical relevance).[11] Visible light-sensitive hydrogels were synthesised by incorporating a light-sensitive chromophore to the hydrogel. When light with an appropriate wavelength applied to the gel, the chromophore group will absorb light, which is then appeared in a local heat by radiationless transitions. When increasing the temperature of the gel, the swelling behaviour will be changed, which undergoes to thermo-sensitive hydrogels principle. The intensity light and the concentration of chromophore increase proportionally with increasing the temperature.[11]

Since the volume of gel changes *via* the mechanism of the visible light by inducing temperature changes with photosensitive molecules, infrared irradiation can be employed to elicit hydrogel response in the absence of the chromophores.[11] This approach is valuable due to the high infrared light absorbency of water.[11]

For clinical application, light-sensitive hydrogels are specifically important due to special advantages over other fields. Light-sensitive gels may be applied in the enhancement of photo-responsive artificial muscles, drug delivery system, switches and memory devices.[11]

One of the main limitations of the light-sensitive hydrogel is that the action of responding gel to light is immediate, while the rate of reaction to stimulus in such action is still slow.[11] In many cases, it is necessary to generate heat by converting light energy into thermal energy to lead to restructuring chains of the polymer upon temperature change.[11] Another challenge is chromophore, which is bonded to the backbone of the polymer *via* covalent-linking lead to leach out during swelling–deswelling process.[11]

Hydrogels can be formed from polyethylene glycol (PEG) and its derivatives polyethylene glycol diacrylate (PEGDA) and ethylene glycol dimethacrylate (PEGDMA) (**Scheme 1**). Polyethylene glycols (PEGs) are organic compound prepared from ethylene oxide, which consist of a repeating unit of –(O-CH_2-CH_2). Synthetic PEGs can be hydrophilic oligomers or polymers, which are available in a board range of molecular weight of PEGs.[12]



Scheme 1. Chemical Structures of acrylate and methacrylate functionalised PEG derivatives.[12]

Hydrogels based on PEG are commonly used in a variety of biomedical applications due to their unique properties. Most of these frequently used PEG gels are prepared either by physical or chemical interaction, which are relatively stable structure of hydrogel with appropriate physical and chemical characteristics such as incorporation of degradable linkers into the network crosslinks.[12]

Supramolecular polymer based hydrogels represent another approach to developing gels for biomedical applications.[13] Several unique properties of hydrogels based on PEG make them appropriate for exploring the system of cell response to specific changes in scaffold hydrogel properties (particularly because PEGs do not absorb proteins) and therefore require conjugation of specific biomolecules.[13] Hydrogel scaffolds can adsorb bioactive proteins from serum or plasma, although if they are not crosslinked into the hydrogel structure their presence is transient.[13] The degradation rate of PEG hydrogels occurs by hydrolytic cleavage of moieties in the structure of the gels (e.g. ester links between the backbone of PEG polymer and their termini or crosslinking units).[13]

Hydrogels have received considerable attention for medical applications, particularly in lenses, drug delivery, tissue engineering, regenerative medicine, cellular immobilisation, biosensors, diagnostics and bio-separations due to their unique characteristics involving high permeability, high water content biocompatibility and mechanical properties.[1,14]

Polyethylene glycol (PEG)-based hydrogels benefit from PEGs lack of toxicity, warter solubility.[15] The chemistry of the termini are often hydroxyl (-OH) which can be conjugated with crosslinkable groups and bioactive molecules.[16] Acrylate and methacrylate PEG derivatives can be light-cured and being convenient materials to obtain hydrogels with microstructures, and complex shapes with various gradients

crosslink density. For variable applications, PEG-based hydrogels are typically fabricated from prepolymers with molecular weights ranging from 2000-10,000 Da, in order to get large protein-permeable polymer networks.[16]

O-nitrobenzene and its derivatives can be utilised as crosslinkers for photodegradable materials or as linkers in bioconjugates or in block copolymers. ONB group incorporated as crosslinkers into materials in order to control their characteristics, degradation, and potentially drug release upon the application of light.[17,18]

This study aims to develop properties of hydrogels responding to light for potential biomedical applications. In this paper, hydrogels based on polyethylene glycol diacrylate (PEGDA) solution have been investigated. This hydrogel was prepared with different concentrations of non-photoactive and photoactive crosslinkers, respectively. The photocleavable unit which is incorporated to hydrogels based on conjugating PEG derivatives to O-nitrobenzene diols has been synthesised to enable the development of photoactive gels for biomedical applications.

4. Experimental section

4.1. Materials

Polyethylene glycol diacrylate (PEGDA 6 KDa) and ethylene glycol dimethacrylate (EGDMA 198.22 g/mol) were purchased from Sigma-Aldrich. Thermal initiator benzoyl peroxide, 97% (dry weight) was supplied by Alfa Aesar. Tablets of phosphate-buffered saline (PBS) were purchased from Sigma-Aldrich. *α,ω*-Bis{2-[(3-carboxy-1-oxopropyl)amino]ethyl}polyethylene glycol was supplied from Sigma-Aldrich. 2-nitro-p-xylylene glycol Combi-Blocks. 4-(Dimethylamino)pyridine, N,N-Diisoproyl carboiimide (DIC), and N-methyl-2-pyrrolidone(NMP) were purchased from Sigma-Aldrich.

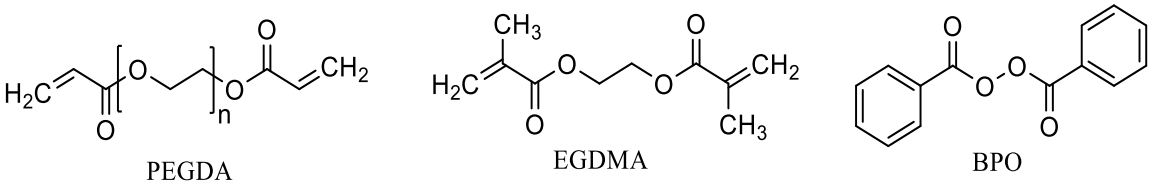
4.2. Methods

Photoactive and non-photoactive hydrogels were formed varying quantities of crosslinkers respectfully. For the non-photoactive gels (low XL): PEGDA (5 ml), EGDMA (0.1 ml), BPO (0.04g). Non-photoactive gels (high XL): PEGDA (5 ml), EGDMA (0.5 ml), BPO (0.04 g). photoactive gels. For the photoactive gels (low XL): PEGDA (5 ml), photolabile crosslinker (0.1 g), BPO (0.04 g). Photoactive gels (5 %): PEGDA (2.5 ml), photolabile crosslinker (0.25 g), BPO (0.02 g).

4.2.1. Preparation techniques of non-photoactive PEGDA Hydrogel with low XL and high XL of non-photolabile crosslinkers

Polyethylene glycol diacrylate (PEGDA) with average molecular weight (Mn) of 575 Da - 6 kDa was used for this synthesis. In a glass vial with a stirrer bar, PEGDA was added and the glass vial was placed on a hotplate at 60 ^{o}C. When diacrylate melted a non-photolabile crosslinker (EGDMA) was added t and the mixture left to homogenise, after which benzoyl peroxide (BPO, the thermal initiator) was added, and the mixture was injected into circular molds of 5 mm diameter and 1 mm height. The molds were

placed in the oven at 90 ºC for 3 hours. Then, the molds were left to cool to room temperature. To remove non-crosslinked components, the gels were washed extensively with DI water for 3 days. Chemicals used are depicted in **Scheme 2**.

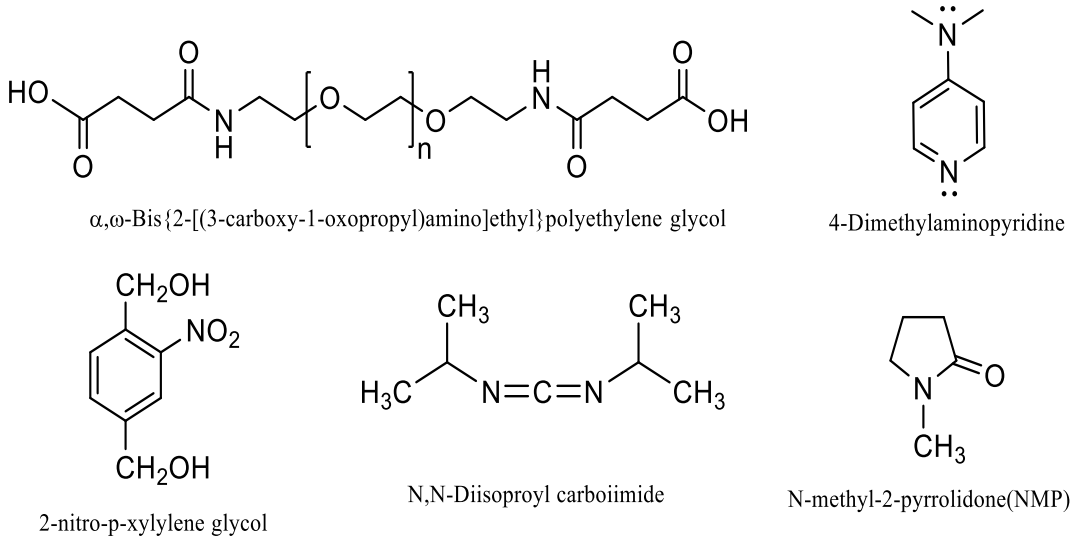


Scheme 2. Chemicals used for the preparation of non-photoactive hydrogel based on PEGDA.

The same procedure was repeated by replacing non-photolabile crosslinker with photolabile crosslinker based on photoactive PEG derivatives.

4.2.2. Synthesis of photoactive PEG derivatives

α,ω-bis{2-[(3-carboxy-1-oxopropyl)amino]ethyl}polyethylene glycol (0.5 g, 0.083 mmol), anhydrous alcohol 2-nitro-p-xylylene glycol (0.012 g, 0.086 mmol) and 4-(dimethylamino)pyridine-DMAP (0.010 g, 0.081 mmol) in *N*,*N*-diisoproyl carboiimide (DIC) (5 mL) were mixed in a dry 250 mL neck round-bottom flasks with stirrer. By the time, the mixture was stirred to become homogenous, 10 mL of N-methyl-2-pyrrolidone (NMP) was gradually added until the solids were completely dissolved. To avoid any undesirable chemical reactions to occur, the reaction was placed under inert atmosphere, stirred for 24 hours at room temperature, and covered with foil (**Scheme 3**). After 24 hours, the mixture was dialysed against deionised water in dialysis membrane (MWCO 3500 Da) for 3 days to remove any organic solvents. Then, the reaction vessel was connected to a vacuum evaporator to evaporate the solvent, yielding a yellow residue. The product was extracted approximately three times by cold ethyl acetate and filtered. The filtrate was combined and evaporated to dryness. The purification of synthesised ester was achieved by silica column chromatography (hexane:ethyl acetate 70:30), and the eluates were detected by TLC (SiO_2, hexane:ethyl acetate 70:30). The product was colourless; therefore, a UV-lamp was used to locate the eluent on TLC plates impregnated with a fluorescent material that glows under ultraviolet (UV) light.



Scheme 3. Chemicals used for the preparation of photolabile PEG derivatives.

4.2.3. Characterization

Fourier transform infrared (FTIR) was recorded on a Cary 630 FTIR (Agilent Technology) spectrophotometer at room temperature in wavelength ranging from 500 to 4000 cm^{-1}. For nuclear magnetic resonance, ^{1}H NMR spectra were recorded in $CDCl_3$ using a Bruker 400 MHz Ultrashield plus NMR spectrometer. Singlet (s), doublet (d), multiplet (m) were used as a notation for ^{1}H NMR spectral splitting patterns. The NMR scanning is running in the range of 0–12 ppm. UV-vis spectra for LED irradiation were recorded with a UV-vis (Agilent Technologies) spectrophotometer and wavelengths were collected within a range of 300–600 nm at room temperature.

5. Result and Discussion

Photoactive and non-photoactive hydrogel was prepared with low XL and high XL of crosslinkers, respectfully (**table 1 & 2**).

Table 1. Formulation of non-photoactive PEGDA hydrogel with non-photolabile crosslinkers.[19]

Non-photoactive hydrogel	low XL	high XL
PEGDA	5 ml	5 ml
EGDMA	0.1 ml	0.5 ml
BPO	0.04 g	0.04 g

Table 2. Formulation of photoactive PEGDA hydrogel with photolabile crosslinkers.

photoactive hydrogel	low XL	high XL
PEGDA	5 ml	2.5 ml
BPO	0.04 g	0.02 g
Photolabile crosslinker (PEG derivatives)	0.1 g	0.25 g

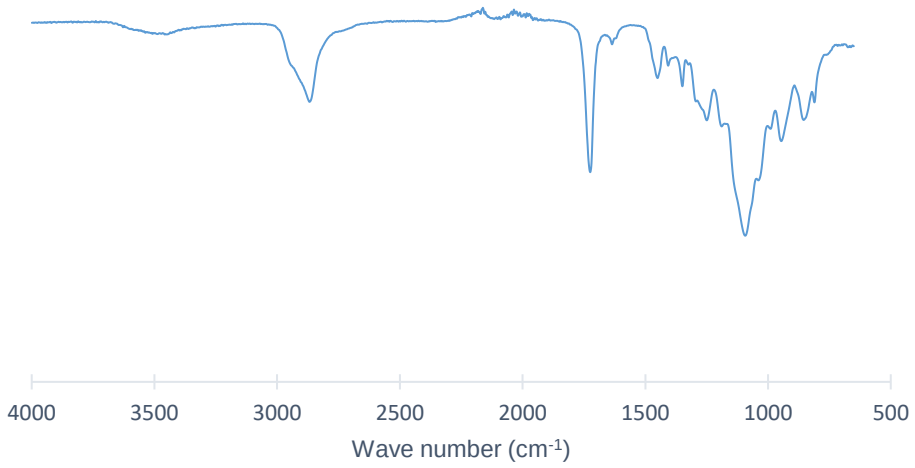


Figure 4. FT-IR of the non-photoactive PEGDA hydrogel.

The FT-IR spectrum of the non-photoactive PEGDA hydrogel is shown in **figure** . Absorption of the carbonyl groups was observed at 1724 cm^{-1} and C-H and CH_2 bonds at 2877cm^{-1}. The absorption band at 1633 cm^{-1} was attributed to aliphatic double bonds C=C. Additionally, the characteristic absorption peaks of ether and ester at 1088 cm^{-1} and 1719 cm^{-1}, respectively.

5.1. Swelling study

The swelling test was studied by measuring the mass of dry and swollen hydrogel using BM-20 microbalance. The swelling ratio based on mass (Q_M) is measured experimentally on the PEGDA hydrogels for calculating the volumetric swelling ratio (Qv) of the hydrogels in PBS. The swelling ratio Q_M is measured *via* dividing the mass of swelling in solvent by the dry mass. The results of the swelling ratio (Q_M) of the gels were calculated using the following equation;

$$Q_M = M_s/M_d \quad (1)$$

Where, *Q*v is the swelling ratio, M_s,M_d are the masses of materials in the swollen and dried states, respectively. The equation (1) was applied to calculate the volumetric swelling ratio, Qv in which ρ_P is the mass-weight average density of the dry hydrogels (PEGDA = 1.12 g/mL at 25 ^oC; EGDMA =1.051 g/mL at 25 ^oC) and ρ_S is the density of the solvent (1 g cm^{-3} for PBS). The volumetric swelling ratio *Qv* can be calculated by utilizing the formula:

$$Qv= 1+ (\rho_P /\rho_S)(Q_M – 1) \quad (2)$$

The cylindrical hydrogel disks with 5 mm diameter and 1 mm thickness were totally dried in vacuum oven. The dried gels were weighed and immersed in 10 mL phosphate-buffered saline (PBS) (pH 7.4) at room temperature for three days for reaching equilibrium.

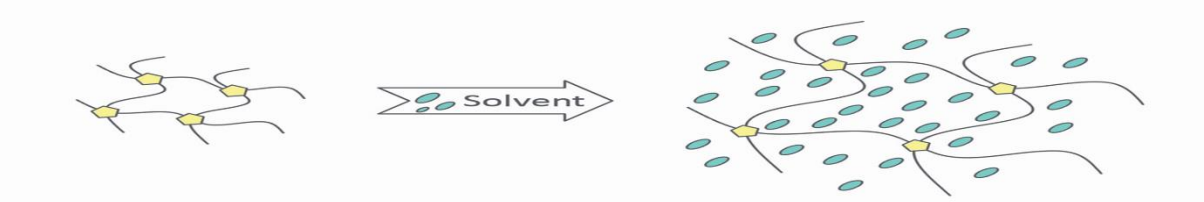


Figure 5. Illustration the difference in the structure of dry and swelled gels. The network of hydrogels swelling when immersed into a solvent.

The effect of photolabile and non-photolabile crosslinking concentration on the swelling of hydrogels based on PEGDA was summarised in table 3.

Table 3. Gel preparation when swollen in PBS. Gels analysed were discs 5mm in diameter and 1 mm thick. Volume of solvent in disc of gel (µl) calculated by mass differences in swelled and dry states.

Property	**non-photoactive crosslinker (low XL)**	**non-photoactive crosslinker (high XL)**	**photoactive crosslinker (low XL)**	**photoactive crosslinker (high XL)**
QM	1.46 ± 0.64	1.43 ± 0.07	1.60 ± 0.01	1.41 ± 0.09
Qv	1.12 ± 0.19	1.06 ± 0.10	3.91 ± 0.36	3.69 ± 0.19
Volume of solvent in disc of gel (µl)	57.91 ± 3.20	17.24 ± 1.13	43.30 ± 2.59	8.05 ± 0.30

Increasing the concentration of crosslinker resulted in a decrease in the PBS absorbency of the gel composite. The capacity of swelling is decreased with increasing concentration of crosslinker due to the increase of crosslinks restricting the movement of polymer chains.

The photocleavage of ortho-nitrobenzyl (o-NB) derivatives which absorb light in the 320–410 nm wavelength are mostly utilised as photodegradable gels. The synthesis of photodegradable gels required the preparation of chromophores consisting of two reactive groups; first group for selective conjugation to a hydrogel chain-end and the other should contain functional group for subsequent polymer cross-linking such as azide or acrylate which able to polymerize and alter the properties of the light absorption as well as reduce the efficiency of degradation.

The spectrum of FTIR (figure 6) shows the presence of the aromatic nitro (C-NO_2) with the characteristic absorption peak at around 1502 cm^{-1} (asymm N-O stretches), and 1300 cm^{-1} (sym N-O stretches). Additionally, the FTIR spectrum would confirm the presence of the carbonyl of ester and amide group bands which appears at 1650 and 1700 cm^{-1}. The figure 6 also shows the isolated H (meta-disubstituted) peak at around 896 cm^{-1}, the peak at around 809 cm^{-1} to the two adjacent H of the (para-substituted) on aromatic ring, additionally, the peak at 2865 cm^{-1} corresponds to the CH_2. The peak of C-O stretch appears at 1070 cm^{-1}.

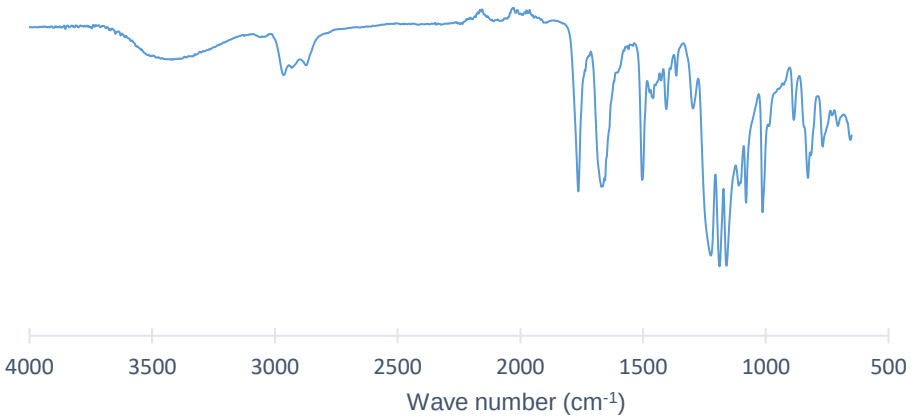


Figure 6. FT-IR of the photoactive PEG derivatives.

^{1}H NMR data (figure 7) confirmed the identity of the product with peaks at 2.00 and 2.40 ppm (t, J = 2.3, O=C-CH_2-CH_2-C=O) groups connected as ester and amide, respectively, 3.42 and 3.57 ppm (t, J = 3.5, NH-CH_2-CH_2-O), 7.18 ppm (d, J = 7.2, AR-H, ortho-coupled) and 7.26 ppm (d, J = 7.2, AR-H, ortho-coupled) confirmed the presence of the aromatic moiety. The presence of CH_3-COO on both terminals of the structure could be also confirmed by the presence of 2 methyl singlets at 1.15 and 1.16 ppm.

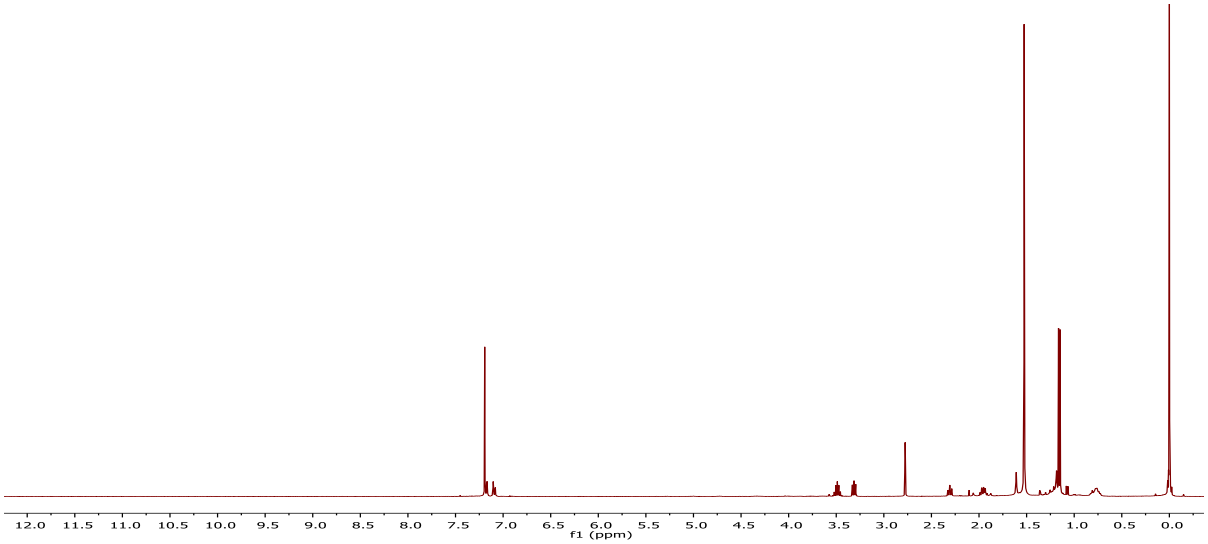
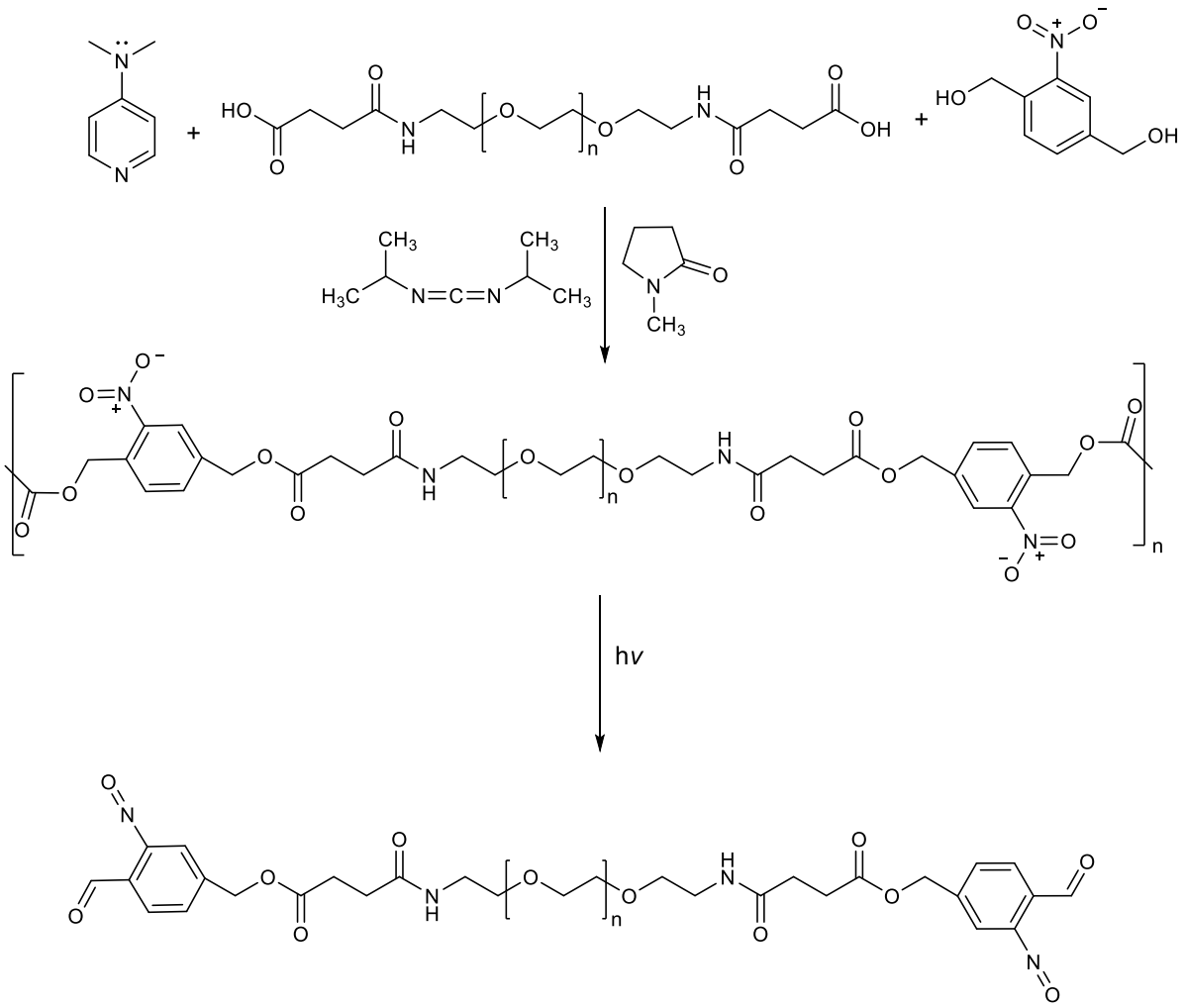


Figure 7. ^{1}H NMR spectra of photoactive PEG derivative.



Scheme 4. Synthesis of the photoactive PEG derivatives.

6. Conclusion

This chapter demonstrated the successful preparation of hydrogels based on PEG with potential for drug delivery systems. The difference in swelling characteristics and rates based on the difference between the higher and lower concentration of non-photolabile and photolabile (O-NB-based) crosslinkers were investigated for drug delivery systems.

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Appendix B:

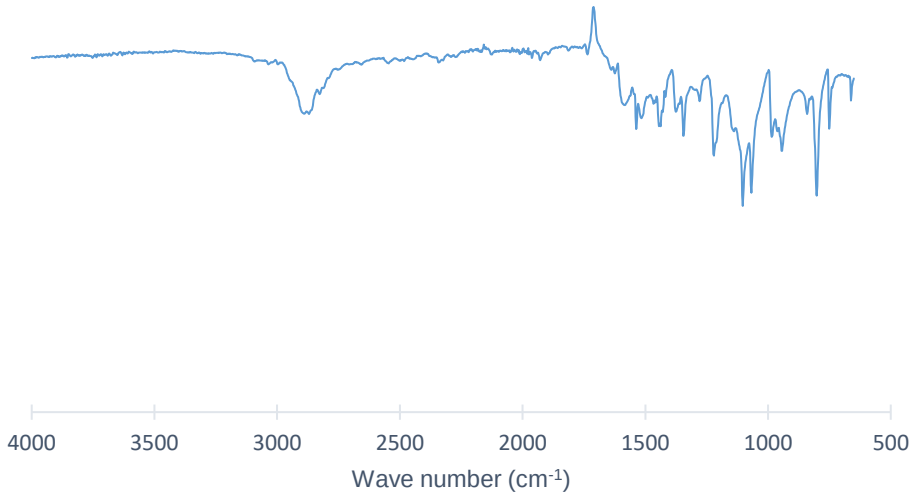

Figure B1. FTIR of *α,ω*-Bis{2-[(3-carboxy-1-oxopropyl)amino]ethyl}polyethylene glycol.

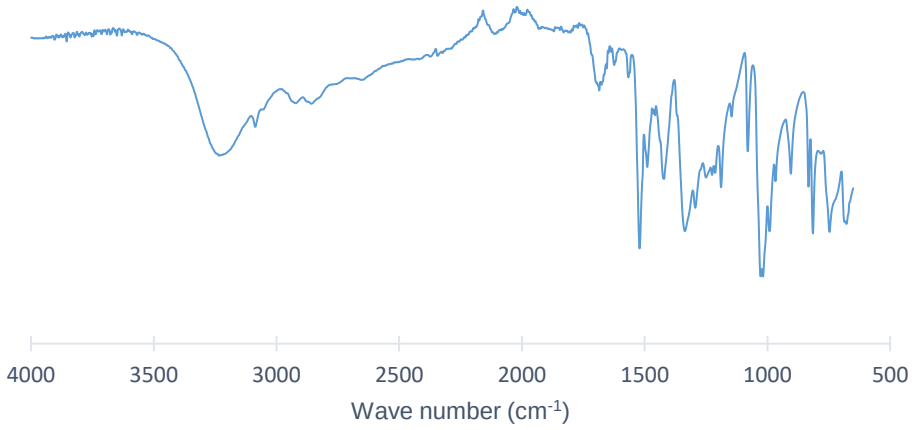

Figure B2. FTIR of 2-nitro-p-xylylene glycol.

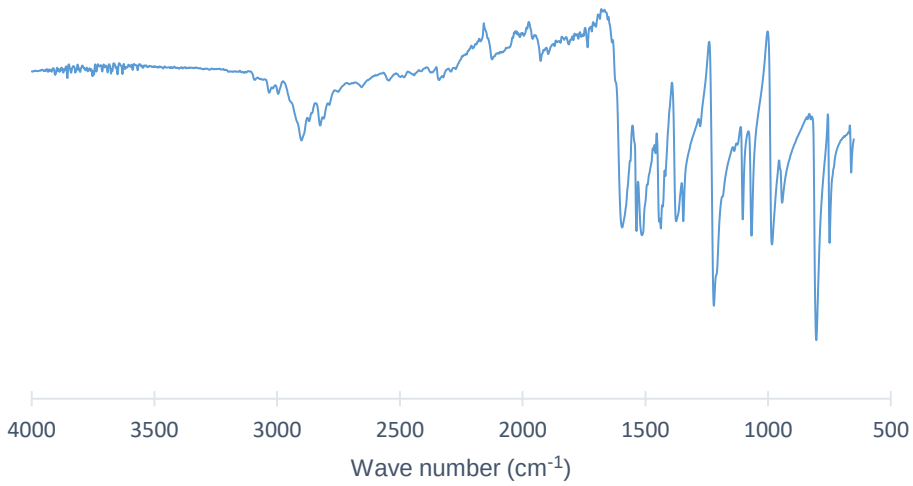

Figure B3. FTIR of 4-(Dimethylamino)pyridine.

Chapter 3. Physicochemical characterisation of degradable photosensitive hemin-polyethylene oxide polymeric micelles

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Abstract:

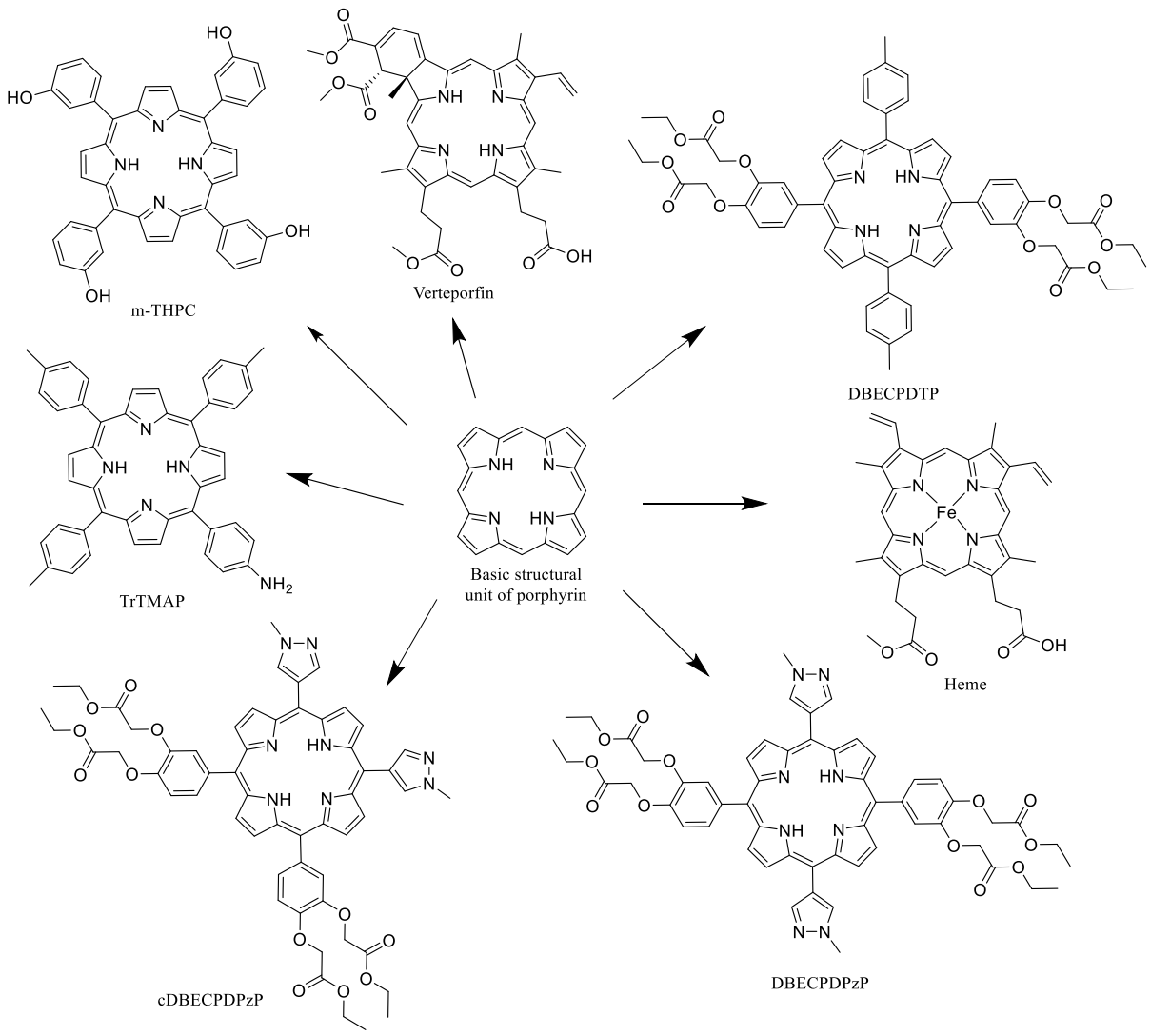
A copolymer based on hemin-polyethylene oxide (H-PEO) was successfully prepared. Hemin grafted to polyethylene oxide is utilised as a photo-triggered switch for the controlled release of encapsulated anti-inflammatory drug (dexamethasone 21-phosphate disodium). It can also be utilised in the area of photodynamic therapy where the copolymer is considered as a photosensitizer which responds to laser irradiation. Light-sensitive changes such as dispersion in PBS will be explained using UV-vis. Additionally, H-PEO copolymer micelles were characterised after dialysing upon UV–vis spectroscopy at different times.

Keywords: hymen; polymeric micelles; photosensitizer; photopolymeric.

1. Introduction

Many clinical reports have indicated that photosensitive polymeric micelles are promising and attractive due to their ability to deliver drugs to target sites with high spatial and temporal precision. In the areas of therapeutics and diagnostics, porphyrins and their derivatives are used as photosensitizers in photodynamic therapy (scheme 1).[1]

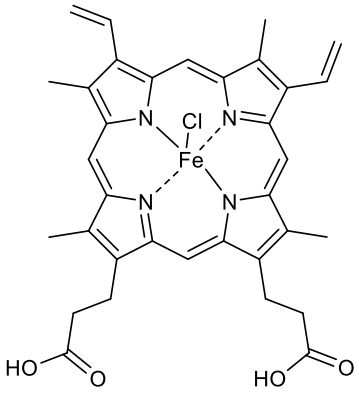
Porphyrins are heterocyclic organic molecules that absorb light. Porphyrins and their derivatives are widely utilised in photomedicine due to their excellent photophysical and photochemical properties.[1,2] Porphyrins can be exploited in radiation treatment, fluorescence imaging and boron neutron capture therapy.[2] In addition, porphyrin derivatives (i.e. tetrapyrrole structures) are the basis of the majority of photosensitizers used in photodynamic therapy of cancers. These molecules accumulate in tumour tissues and absorb light wavelength ranges from 600 to 800 nm due to the conjugated systems in their chemical structure.[3,4]



Scheme 1. Chemical structure of some clinically utilized photosensitive based on structure of porphyrin.

The photosensitive polymeric micelles based on hemin-polyethylene oxide (H-PEO) are promising and attractive owing to the controlled drug release into their target with improved drug accumulation in tumour tissues at high spatial and temporal precision.[1] Loading and photo-triggered release of optional drug *in vitro*, in addition to light-stimuli, which is used to degrade linkages, are very important for enhancing the efficiency of cancer therapy.[1]

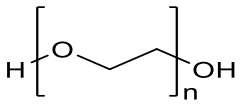
The chemical structure of hemin comprises two classes of reactive functional groups; vinyl and propionic acid moieties.[1] The latter groups readily react with hydroxyl groups (-OH) in hydrophilic polyethylene oxide, then vinyl groups could be introduced into polymer chains for processing the crosslinking. Polymeric micelles based on hemin exhibit excellent performance in oxidation and better stability over wide pH and temperature range.[5]



Hemin

Figure 1. Chemical structure of hemin.

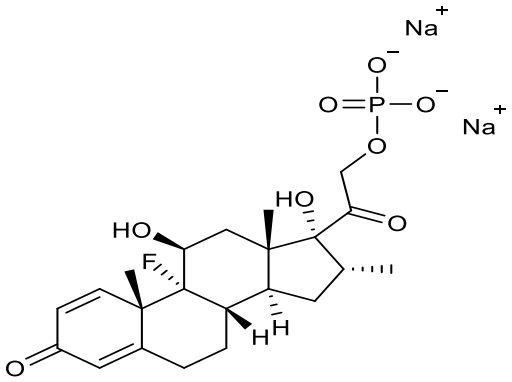
Polyethylene oxide (PEO) is linear polymer with hydroxyl functional groups that possesses high viscosity even at low density. It is soluble in water as well as various organic solvents. It is available in a wide range of molecular weights but generally possesses a high molecular weight compered to PEG. Due to its low toxicity, PEO can be used in variable applications. In a variety of polymeric micelles, PEO represents the hydrophilic segment.[6]



PEO

Figure 2. Chemical structure of PEO.

Dexamethasone sodium phosphate is an organic salt form of dexamethasone. In clinical applications, dexamethasone sodium phosphate is widely used as synthetic adrenal corticosteroid hormone with potent anti-inflammatory properties.[7]



Dexamethasone sodium phosphate

Figure 3. Chemical structure of dexamethasone sodium phosphate.

This chapter describes the preparation of the micelles and the characterisation of novel polymeric amphiphiles based on hemin as a hydrophobic segment and PEO as a hydrophilic segment. The physicochemical characterisation of degradable photosensitive H-PEO polymeric micelles has been studied briefly.

2. 2. Experimental section

2.1. Materials

Hemin-polyethylene oxide H-PEO (Mw= 16.654 g/mol), tablets of phosphate-buffered saline (PBS) were purchased from Sigma-Alorich and distilled water.

2.2. Methods

2.2.1. Preparation of hemin-polyethylene oxide micelles

H-PEO micelles were prepared by dialysis method. Briefly, H-PEO (3mg) was dissolved in 1mL of PBS [one tablet of PBS was dispensed in 200 mL of distilled water to obtain a 2.7 mM KCl, 137 mM NaCl, and 10 mM phosphate buffer solution (pH 7.4 at 25 °C) MW=10]. Then, 1 mL of water was added dropwise and the mixture was stirred until totally dissolved. The mixture was then dialysed against deionised water (Fisherbrand^{TM} Regenerated Cellulose, MWCO 3500 Da) for 72 hours.

2.2.2. Loading drug

Approximately 0.06 mg of dexamethasone 21-phosphate was loaded into H-PEO micelles by adding 1 mL of distilled water dropwise to PBS solution containing H-PEO. The mixture was stirred at room temperature till completely dissolved, followed by dialysis against deionised water and 10 mM PBS (Fisherbrand^{TM} Regenerated Cellulose, MWCO 3500 Da) for three days in the dark (wrapped in aluminium foil). The dialysis medium was changed more than four times. The concentration of the polymer was 0.3 mg.mL^{-1}.

2.2.3. Characterization

Fourier transform infrared (FTIR) spectra was collected at room temperature by using a Cary 630 FTIR (Agilent Technology) in a range of 500-4000 cm^{-1}. UV-vis spectra of H-PEO was carried out using a UV-vis Thermo scientific^{TM} NanoDrop Spectrophotometer. Spectra were collected within a range of 200–800 nm. Light-emitting diodes (LED) were carried out using a ThorLabs light emitting diodes (LED) DC4104 Driver system with a LED (365) nm in light proof container (outdoor plastic garden shed manufactured by Keter).

3. Result and discussion

H-PEO was obtained by coupling hemin to PEO *via* esterification reaction (a Steglich reaction analogous to that described above). Polymeric micelles may serve as perfect nanocarriers for delivering insoluble drug to target tumours. As an attempt to prepare the H-PEO micelles, 3 mg of H-PEO was dissolved in 1 ml of PBS.

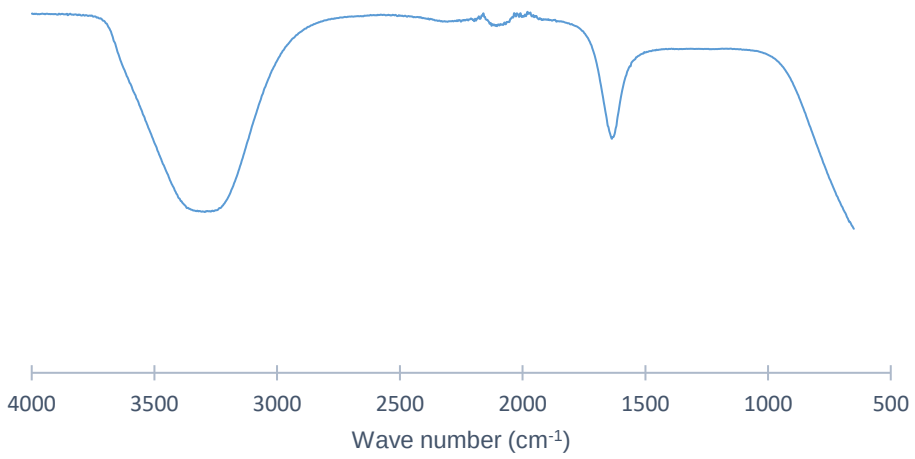


Figure 4. FTIR of H-PEO.

Figure 4, shows the main characteristic absorption peaks of H-PEO after dialysis that represent by bond of N-H and C=C at 3300 cm^{-1} and 1620 cm^{-1}, respectively.

H-PEO could be self-assembled into micelles in aqueous media. In PDT, hemin is serving as a photosensitizer and photo-triggered multimodal antitumor action. Typically, hemin is not soluble in most organic solvents, acidic and neutral aqueous media. However, in alkaline water, it possesses relatively good solubility due to the presence of two carboxy groups on the hemin.[3]

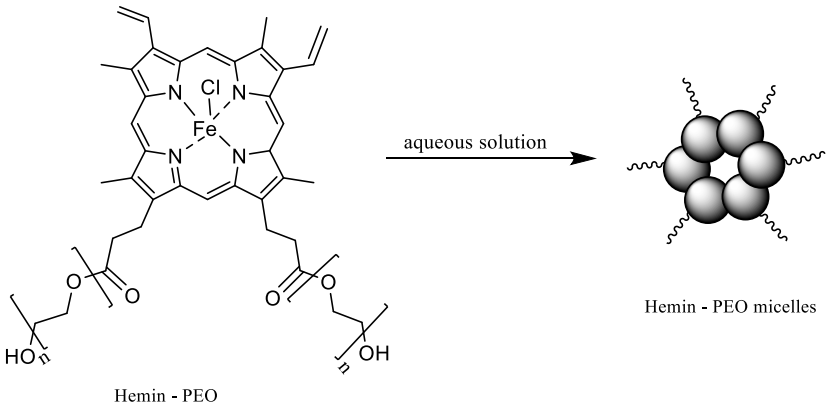


Figure 5. Photopolymeric micelles of H-PEO

Hemin is hydrophobic and forms the core inside polymeric micelles in aqueous media, enabling the encapsulation of poorly water-soluble drugs, with a shell of hydrophilic PEO.

The destruction of the porphyrin ring leads to release of the drug into the target site. UV-vis spectra were used to investigate the destruction of the of porphyrin ring in hemin upon irradiation with light (405 nm, 200 mW).

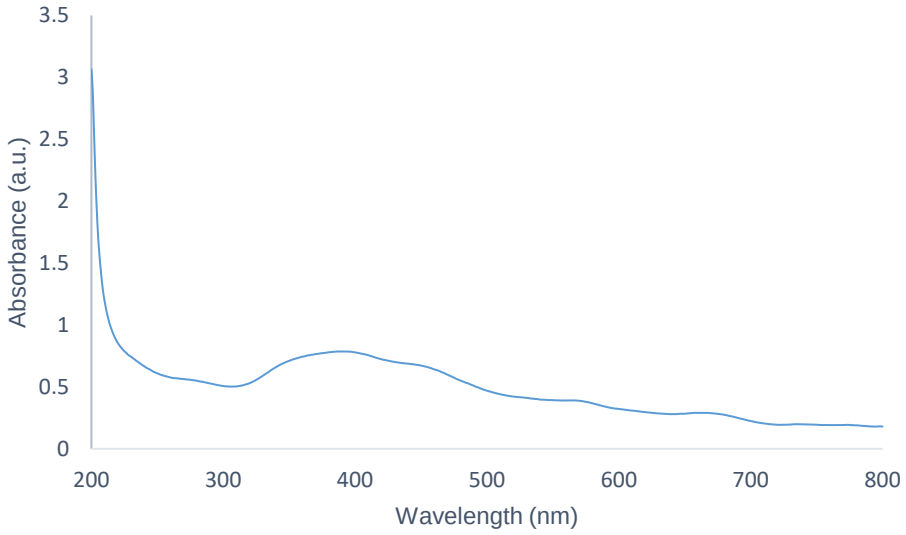


Figure 6. UV-vis spectra of PEO-hemin (3 mg/ml in PBS).

According to figure 7, increasing the irradiation time decreases the absorption peak intensity of hemin. The destruction of hemin results in damaging of large π bonds from the ring of porphyrin.

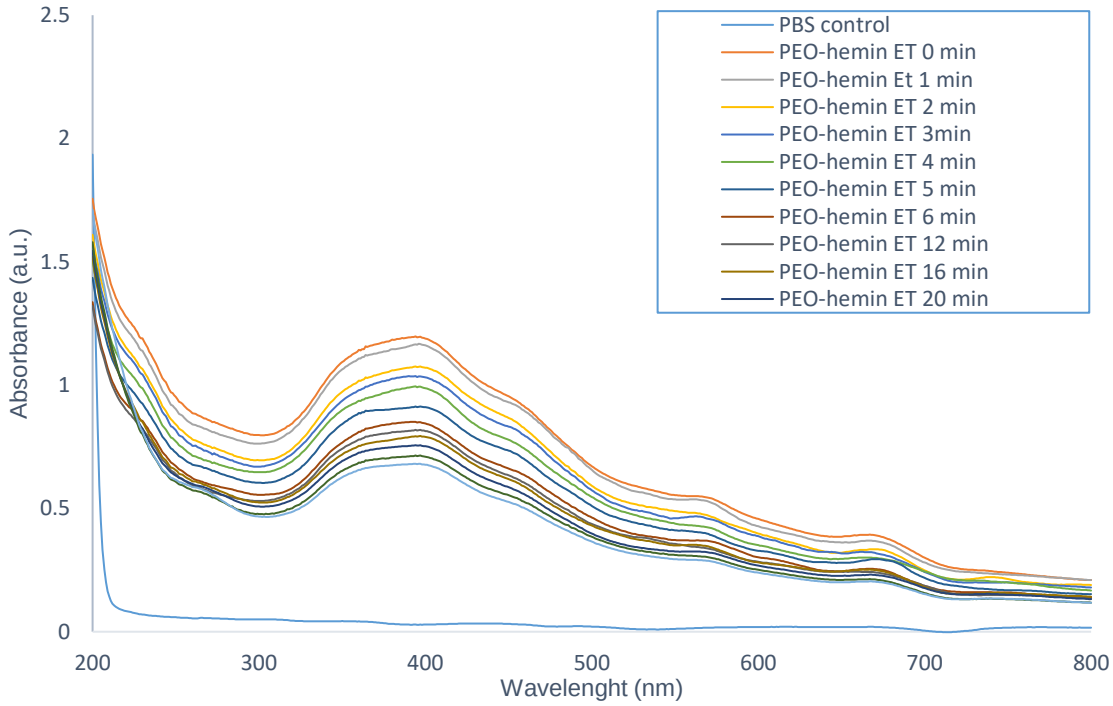


Figure 7. UV-vis spectra of PEO-hemin (3 mg/ml in PBS) after dialysis and irradiation with 365 nm light for various times (ET= elapsed time).

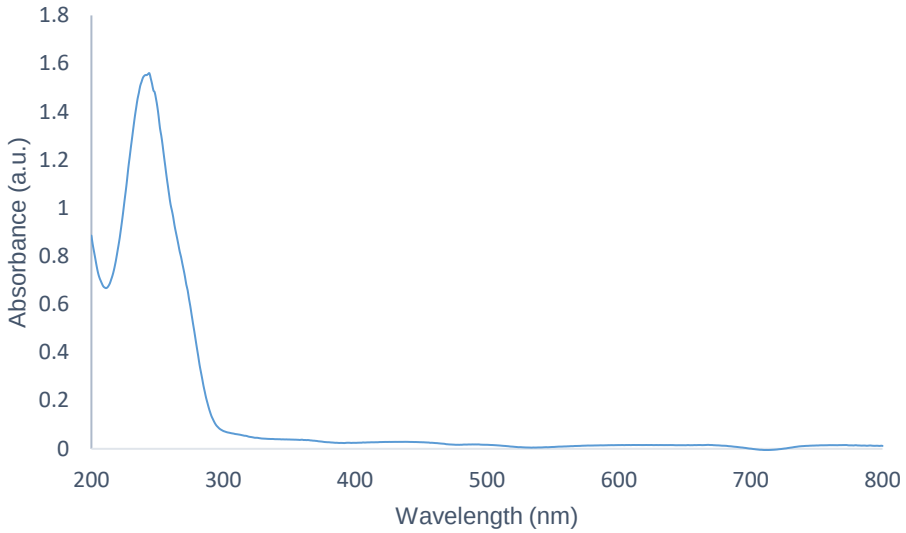


Figure 8. UV-vis spectra of dexamethasone 21-phosphate disodium (0.06 mg/ml in distilled water).

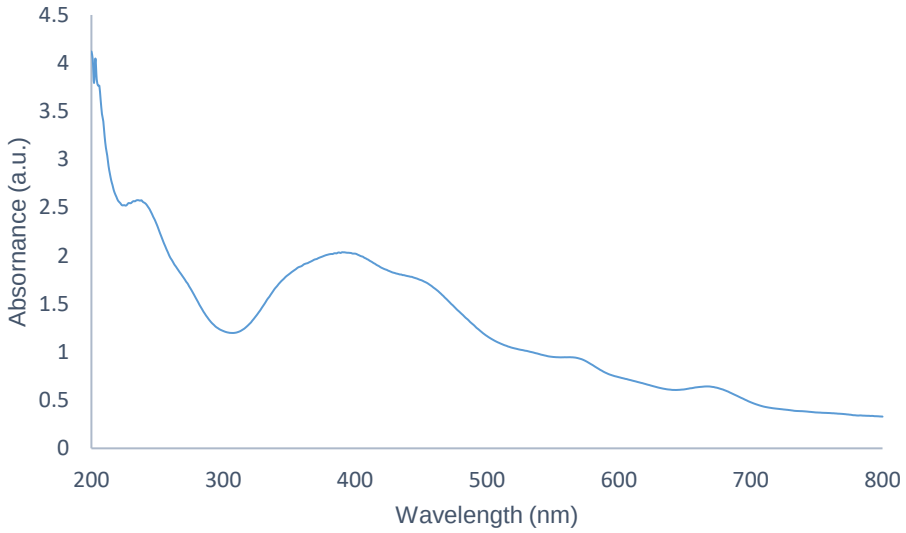


Figure 9. UV-vis spectra of H-PEO loading dexamethasone 21-phosphate disodium.

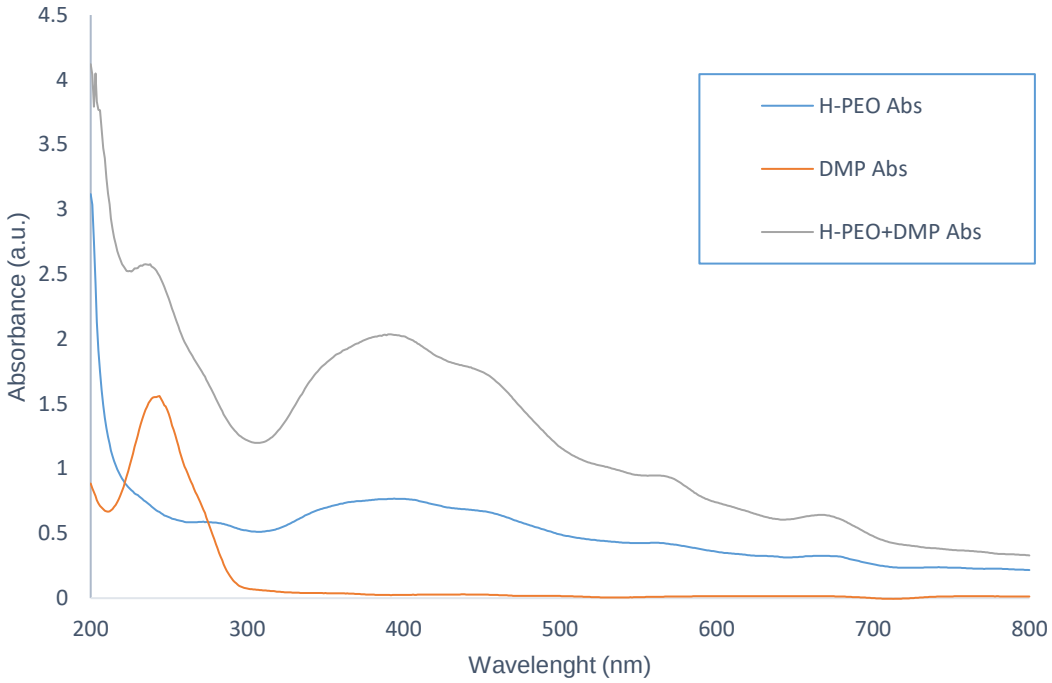


Figure 10. UV-vis spectra of H-PEO, DMP and H-PEO loading DMP.

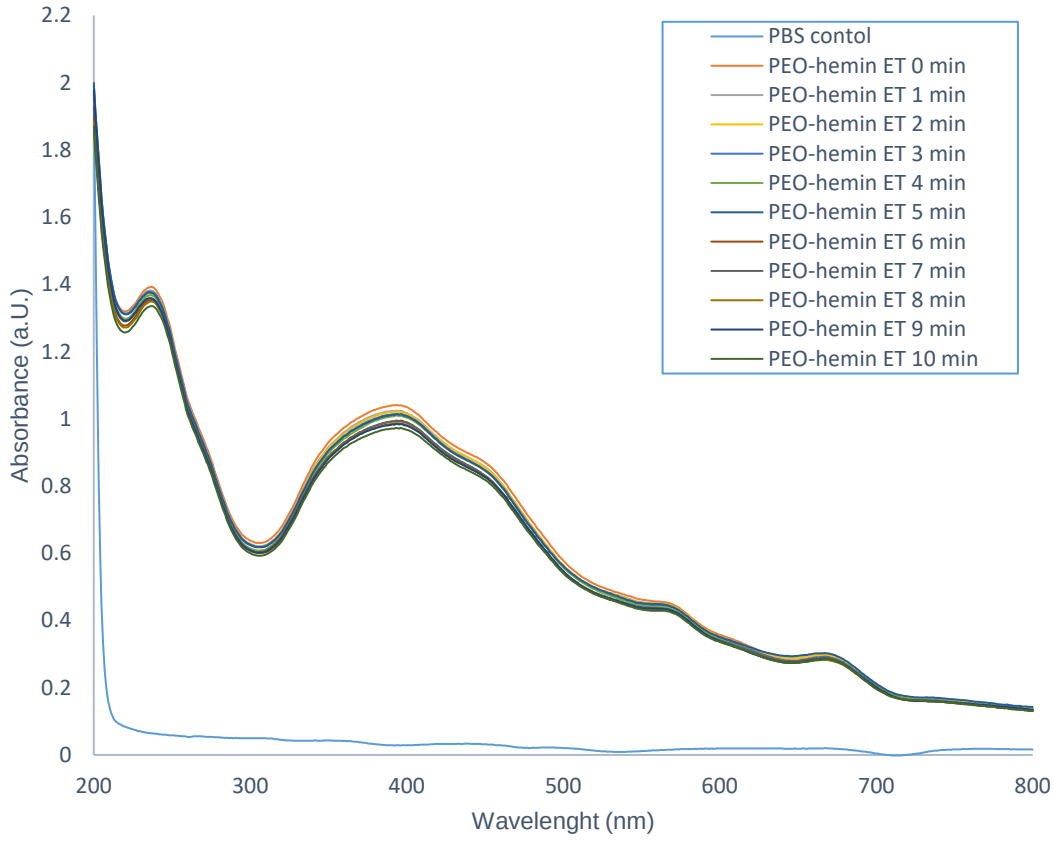


Figure 11. UV-vis spectra of H-PEO loading dexamethasone 21-phosphate disodium after dialysis.

4. Conclusions

There are prospects for the development of polymeric micelles based on H-PEO for drug delivery system applications (which show changes in dispersion in PBS upon light irradiation, i.e. degradation was increased with increasing the time of irradiation). A significant amount of further physiochemical analysis will be necessary to generate light-responsive drug delivery systems capable of delivering drugs to patients in a highly reproducible and patient specific fashion.

5. References

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Appendix C:

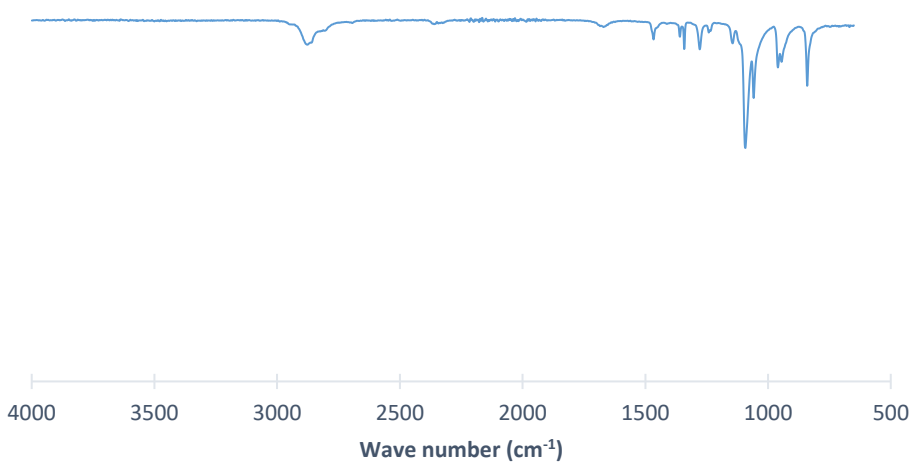

Figure C1. FTIR of pure H-PEO.

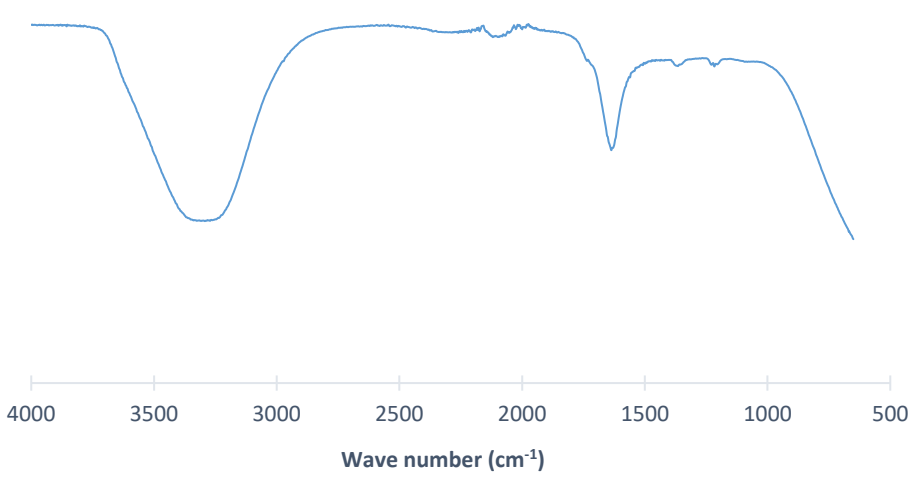

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Figure C2. FTIR of H-PEO loading dexamethasone 21-phosphate disodium after dialysis.

Conclusion

The objective of this thesis is to investigate the synthesis of novel photoresponsive polymers for biomedical applications. For exampe photocleavable polymers that are able to form micelles, which potentially act as nanocarriers of the poorly water soluble drugs (e,g. Cabozantinib, Nintedanib, Curcumin, Paclitaxel) to tumour sites, delivering the drugs in response to light.

Methodology 1) Developing of photoactive biocompatible drug vehicles was achieved by modifying hyaluronic acid with photoactive hydrophobic moieties, which may be converted to hydrophilic moieties upon exposure to appropriate light irradiation. Irradiation with light stimuli can induce the changes in micelle structure and release payload at the target tumour. Degradable polymers are popular for DDSs, generally containing labile bonds such as ester, amide, and anhydride bonds that are prone to hydrolysis or enzymatic degradation. HA binds to CD44 which is overexpressed on the surface of some cancer cellssuggesting there is long term potential for such systems to deliver drugs in a targeted fashion.

Methodology **2)** Hydrogels have received significant interest due to their properties, particularly in biomedical applications (e.g. drug delivery systems, tissue scaffolds for regenerative medicine). A variety of hydrogels were prepared (with either photocleavable or non-photocleavable crosslinkers) and difference in swelling rates studied. The route of photoactive hydrogel based on a combination of the PEG diacid derivatives and O-nitrobenzyl diols were synthesised and incorporated to gels.

Methodology **3)** Photosensitive copolymers based on Hemin-PEO were prepared to form micelles in water. The degradation of the porphyrin ring of hemin upon exposure to light was studied. Such micelles have potential to serve as nanocarriers for delivering insoluble drugs to target tissues.