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Ultrasonic monitoring of drug loaded Pluronic F127 micellular hydrogel phase behaviour

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ABSTRACT

Pluronic hydrogels composed of PEO–PPO–PEO tri-block copolymers have received a lot of attention for their applicability to drug delivery. These systems can be injected into the body in a liquid form and then, in response to temperature changes, self-assemble into nano-sized micelles which ultimately aggregate to form a gel. The phase behaviour and effectiveness of Pluronic hydrogels as drug carriers is affected by the local thermal and ionic environment which is likely to be different from patient to patient. There is a current need for in vivo techniques to study the phase behaviour of Pluronic hydrogels and this work demonstrates an ultrasound approach for the study of drug loaded Pluronic F127 hydrogels. Ultrasound velocity and attenuation were both found to change with temperature and through validation with fluorescence spectroscopy it was determined that the temperature dependent micellation transition in the Pluronic solutions could be identified through relative changes in ultrasound velocity and attenuation as a function of temperature. This phase transition was more clearly detected through examination of the first and second derivatives of both ultrasound parameters with respect to temperature. Further this work demonstrates for the first time to our knowledge ultrasound characterisation studies on drug loaded Pluronics.

1. Introduction

The use of hydrogels in biomedical applications is growing, particularly due to advances in polymer synthesis that produce stimuli sensitive materials which respond to environmental changes such as temperature and pH. Hydrogels are water-swollen polymeric networks that contain approximately 60% to 99% water, yet maintain the structural integrity of a solid due to the presence of cross-links [1]. This high water content coupled with their soft, rubbery nature and typically high permeability makes hydrogels attractive biomaterials [2]. Recently there has been great interest in using hydrogels as vehicles for delivery of drugs [3] and cells [4–6] into the body for therapeutic purposes. In particular, stimuli sensitive hydrogels that can be injected into the body in a liquid state and subsequently form a gel once in the body are favourable due to the minimally invasive route of administration [7].

Hydrogels can be produced in a number of ways and can be classified by the type of cross-links forming the polymeric network. For example, cross-links can be chemical e.g. covalent bonds, physical e.g. secondary bonds including hydrogen bonding, van der Waals forces, crystal junctions, micellar packing, etc. or hybrid e.g. a combination of chemical and physical cross-links. The type of cross-link affects hydrogel degradation, mechanical properties, permeability and binding affinity [1] and thus its suitability for use in different applications.

Hydrogels composed of amphiphilic block co-polymers, which gel by micellar packing in response to temperature changes, have received a lot of attention for their applicability to drug and cell delivery [8–11]. These systems can flow freely in their liquid form and then, in response to temperature changes, self-assemble into nano-sized micelles and fibres which ultimately aggregate to form a gel [7]. Thus, prior to administration, and while the system is in the liquid phase, drugs and/or cells can be mixed into the solution. As temperature rises the therapeutic agents can be encapsulated in the forming micelles and eventually in the gel structure itself. This capability enables water-insoluble or poorly soluble drugs as well as labile molecules such as proteins and peptide drugs to be effectively solubilised [12].

Micellar hydrogels composed of PEO–PPO–PEO tri-block copolymers, also known as Pluronics [7], have been widely studied for drug delivery applications with some formulations being approved for therapeutic use by the United States Food and Drug Authority (FDA) [13]. Medical applications of these hydrogels include use in the treatment of wounds [14], transdermal delivery of protein and peptides (insulin, urease, bone morphogenic protein and growth factors) [15], delivery of drugs in ophthalmic applications [16], prevention of postoperative adhesions [17] and the delivery of chondrocytes to promote cartilage regeneration [18]. Continued work using Pluronic hydrogels have

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focussed on modifying their drug release profile and reducing the polymer concentration required for gelation, for example, by altering the hydrophilicity of polymer side chains.

Due to the thermoresponsive nature of Pluronic hydrogels it is important to characterise their phase behaviour prior to application. This phase behaviour involves a transition from a liquid state in which the polymer chains are dispersed as unimers towards a more complex liquid-like state with polymer micelles and finally on to a gel state due to packing of the micelles [19]. The most widely used Pluronic hydrogel has a formulation with a nominal molecular weight of 12,500 and a PEO/PPO ratio of 2:1 by weight, and is commercially known as Pluronic F127. Extensive studies of the phase behaviour of Pluronic F127 hydrogels have been reported in the literature using techniques such as light scattering, small angle neutron scattering, ^{1}H nuclear magnetic resonance (NMR) spectroscopy, fluorescence spectroscopy, differential scanning calorimetry (DSC) and rheometry [20]. The combined results from all of these techniques have enabled a good understanding of the phase behaviour of Pluronic F127 hydrogels to be obtained. However, as the end application of these hydrogels is in the body it is pertinent that non-invasive methodologies capable of studying the phase behaviour in vivo are developed, particularly as the thermal and ionic environment is likely to be different from patient to patient and in healthy as opposed to diseased states.

In this work the potential of ultrasound techniques to probe the phase behaviour of Pluronic F127 hydrogels is assessed. Ultrasound techniques have been widely used for non-invasive monitoring of physical changes in polymer solutions and gels [21–23], and in a clinical setting for medical imaging [24]. The main benefits of ultrasound characterisation studies are its non-invasiveness, speed of measurements which enable in-process monitoring, ease of use and good sensitivity to physical changes without the use of any contrast agents. A few prior ultrasound studies of Pluronic F127 hydrogels have been carried out, for example the dependence of ultrasound velocity on Pluronic F127 concentration in aqueous solutions was studied using a sing-around technique, in which the average time delay of ultrasound pulses in a closed loop is used to determine velocity [21]. Ultrasound velocity was evaluated as a function of temperature for solutions of Pluronic P85, with a nominal molecular weight of 4600, by Glatter et al. [25]. Measurements were made using a bench top density and sound velocity meter and the observed micellation behaviour was validated with DSC. In these studies plots of the first derivative of velocity as a function of temperature displayed a peak which was reported to be an indication of micelle formation. More recently Cespi et al. have used a high resolution ultrasound spectrometer to assess its potential as a tool to characterise polymer aggregation in Pluronic F127 hydrogels [20]. The dependence of ultrasound relative velocity and attenuation on temperature was presented for a range of concentrations and compared to results of DSC. Further the first derivative of the ultrasound parameters with respect to temperature was also displayed to aid in localisation of the micellation phase transition in the hydrogel. These results agreed with those earlier reported by Glatter demonstrating that ultrasound parameters can be used to indicate micelle formation. These initial ultrasound studies suggested that ultrasound monitoring of responsive polymers should be considered in more detail. Of particular interest is investigation of measurement techniques that could ultimately be applied in a non-invasive way in a clinical setting.

The aim of the work presented here is to implement a robust ultrasound method to study the phase behaviour of Pluronic F127 hydrogels on a larger scale than has previously been performed. Another goal of this work is to incorporate a model hydrophobic drug, pyrene, into the system as both a fluorescent probe to validate micelle formation and to study the uptake of the drug for controlled release applications. This fluorescent method is very sensitive to subtle changes in the microenvironment around the probe [26] and microstructural changes in the micelles at a molecular level [27]. Hence, this approach will provide greater sensitivity than calorimetry studies, which examine bulk properties. Further, this work will for the first time to our knowledge, perform ultrasound studies on drug loaded Pluronics.

2. Materials and methods

2.1. Preparation of samples

Aqueous stock solutions of Pluronic F127 (Sigma Aldrich) were prepared using the cold method, as described in the literature [28]. In practice samples with concentrations of 14%, 16%, 18% and 20% w/w were prepared by adding appropriate amounts of polymer (in flake form) to distilled water. The aqueous Pluronic solutions were then mixed and held at 3 °C for 12 h. The fluorescent probe pyrene (Sigma Aldrich) was then incorporated into the Pluronic solutions. This involved first dissolving the hydrophobic pyrene in methanol, according to the procedure described previously [26] using a concentration of 8.09 mg/100 mL. The pyrene-methanol solution was then sonicated using a Clifton MU 1.5D sonicator until, on visual observation, the pyrene had dissolved. The solution was then left for 24 h in a fridge at 3 °C to promote homogeneous dissolution of pyrene in the methanol. Aliquots of the pyrene-methanol solution were then added to each Pluronic stock solution to produce solutions in a ratio of 5% pyrene-methanol to 95% stock solution. Thus the pyrene loaded co-polymer solutions had final Pluronic F127 concentrations of 13.3%, 15.2%, 17.1% and 19.0% w/w. This phase behaviour of Pluronic F127 solutions as a function of polymer concentration and solution temperature has been well documented [11,19,29]. With reference to the literature, the range of polymer concentrations studied in this work was chosen as it allowed the study of samples representative of solutions that only undergo micellation up to those reaching micellation concentrations sufficient for gelation over the temperatures considered.

2.2. Fluorescence spectroscopy

The phase behaviour of the Pluronic solutions as a function of temperature was studied by a fluorescence technique using the molecular probe pyrene [30]. Pyrene is a hydrophobic probe which is sensitive to the polarity and viscosity of its microenvironment [31]. Of interest to this work is the ability of the pyrene fluorescent probe to form excimers in a concentration dependent way enabling the aggregation in micellar systems to be studied [32]. In this work the fluorescence spectra of pyrene loaded Pluronic solutions were obtained using a Varian Cary Eclipse fluorescence spectrophotometer at an excitation wavelength of 336 nm. For each sample concentration, 1 mL of the pyrene loaded Pluronic solution was placed in a spectrophotometer cuvette and inserted into the thermostated multi-cell holder of the spectrophotometer. The temperature of the sample compartment was then adjusted over a temperature range of 10 °C to 27 °C at steps of 1 °C. Spectra were obtained after a delay of 2 min following each temperature rise to aid equilibration of the sample temperature with that of the compartment. At each temperature the intensities of the monomer band at 385 nm and excimer band at 460 nm were measured and the ratio of the fluorescence intensities of the excimer to monomer determined to provide information about the available hydrophobic volume in the micelle cores, the concurrent changes in the local concentration of pyrene and infer the critical micelle temperature [26,30].

2.3. Tube inversion tests

The critical gelation temperature of the pyrene loaded Pluronic F127 solutions was determined using a tube inversion method. Cylindrical glass bottles of diameter 20 mm and height 50 mm containing 2 mL samples of each concentration were placed in a temperature controlled water bath (Grant Sub Aqua 5) and heated over a temperature range from 17 °C to 32 °C at steps of 0.5 °C. The tubes were allowed at least 15 min at each set temperature point to equilibrate before being removed and tilted. The gelation temperature was defined as the temperature at which the sample did not flow when the tube was inverted [7].