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Liquid crystalline microspheres for 5-fluorouracil specific release

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ABSTRACT

The aim of this study was the synthesis and characterization of swellable microspheres based on fenoprofen and poloxamer. Morphology, average diameter and thermal behaviour of obtained microparticles were studied by scanning electron microscopy, dynamic light scattering, and differential scanning calorimetry. The swelling degree was evaluated at different time intervals and at two pH values. The results revealed that the microspheres swelled better at a pH 6.2, typical of tumour pathologies, than at 7.4, physiological one. Then, microspheres were impregnated with 5-fluorouracil with the aim to increase its bioavailability and reduce its toxicity. The percentage of cumulative amount of 5-fluorouracil released from microparticles was calculated at pH 6.2. Surprisingly, calorimetry and optical microscopy showed the preservation of fenoprofen liquid crystallinity in the obtained microparticles with a transition temperature close to those of typical tumour-sites. The liquid crystallinity of microparticles could be exploited to modulate the release of 5-fluorouracil to tumour site.

1. Introduction

Cancer is one of the most overwhelming diseases of modern society, with an annual frequency of more than 10 million cases [1]. The usual anticancer treatments, e.g. irradiation and chemotherapy, generally cause significant side effects mainly due to toxicity towards normal cells and tissues [2]. In this regard, there has been a flurry of activity towards the development of novel site-specific and stimuli responsive drug delivery systems (DDS) for cancer therapy, in order to maximize the anticancer drug efficacy and avoid their cytotoxic limitations [3,4]. These DDS can deliver chemo-drugs selectively to the tumour area, thanks to various chemical and physical stimuli [5–7].

Hydrogels are crosslinked polymer systems able to retain large amounts of water by swelling. The presence of crosslinking in the polymer matrix makes them insoluble in water. Hydrogel microparticles are micrometre-sized particles that have found many applications in daily life as catalysts, sensors, lubricants, actuators, waste adsorbers, advanced materials, regenerative tissues, imaging tools, fillers, cosmetics, pharmaceuticals, and as DDS [8]. Stimulus-responsive gels offer the opportunity to develop smart drug delivery systems thanks to their ability to swell/shrink in response to external stimuli, such as pH, temperature, ionic strength, external electric/magnetic fields, solvent quality, and enzymes [9–11]. The swelling or shrinking of a microgel is caused by the globule-to-coil or vice versa transition of polymer chains between two neighbouring crosslinking points inside the gel network. For example, the pH-sensitive hydrogels are widely used for administering active drugs irritating to the gastric mucosa by using formulations in a shrunk state at low pH and in a swollen state at high pH values.

Recently, liquid crystalline materials have attracted significant attention for their potential use as DDS with improved properties such as physicochemical stability, drug loading, sustained release patterns, and a reduction in drug leakage [12,13]; in addition to their well-known technological applications [14].

5-fluorouracil (5-FU) is an anticancer agent, analogue of pyrimidine bases, belonging to the family of anti-metabolites. It is widely used alone or in combination with other drugs in the treatment of various malignancies, including cancers of the gastrointestinal tract, breast, ovary, head and neck area [15]. However, 5-FU is characterized by poor bioavailability, low efficacy and high toxicity [16]. Several modalities of drug administration have been investigated to improve 5-FU bioavailability and to reduce the related toxicity [17,18].

Although some progress has been achieved, these approaches have issues of poor drug loading, drug leakage, burst release patterns, and poor physicochemical stability that are unaddressed.

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Fenoprofen is an analgesic, anti-inflammatory and antipyretic nonsteroidal drug that exhibits liquid crystalline properties. Literature data suggest that both fenoprofen sodium and calcium salts can form stable thermotropic mesophases in dehydrated conditions [19–21]. In this mesomorphic state, the director of fenoprofen molecules can align along the direction of an applied external field due to their particular electrical nature [22].

Poloxamers are non-ionic copolymers (triblock) composed of a central hydrophobic chain of polyoxypropylene flanked by two chains of hydrophilic polyoxymethylene [23]. Poloxamers are commonly used polymers in many pharmaceutical applications for their thermogelling properties. In fact, they can be used to increase the water solubility of hydrophobic substances or the miscibility of substances with different hydrophobicity. In addition, they have been evaluated as drug delivery systems and found able to enhance the action of chemotherapeutic drugs. They preferentially target cancer cells, due to differences present in their membranes, and are able to inhibit multi-drug resistant (MDR) proteins, which are responsible for the drug efflux from the cells. Therefore, it is possible to increase the susceptibility of cancer cells to chemotherapeutic agents [24–26].

In addition, literature data report that poloxamers can be utilized for controlled drug delivery applications. Moreover, a designed composition in polymers and poloxamers could lead to the development of DDS with enhanced properties.

This paper deals with the synthesis and characterization of swellable microspheres based on fenoprofen and poloxamer for the controlled and targeted release of 5-FU (Fig. 1).

The obtained microparticles were characterized by scanning electron microscopy, dynamic light scattering, and differential scanning calorimetry. Their swelling degree was evaluated at two different pHs, simulating the environment typical of tumour sites and the physiological conditions, respectively. Then, they were loaded with 5-FU and drug release studies were performed at pH 6.2.

Surprisingly, differential scanning calorimetry and polarizing optical microscopy showed the preservation of fenoprofen liquid crystallinity in the obtained microparticles with a transition temperature close to those of typical tumour-sites. The liquid crystallinity of synthesized microparticles could be exploited to modulate the release of 5-fluorouracil by the application of an external stimulus such as a magnetic field and/or temperature.

2. Materials and methods

2.1. Materials

The solvents such as acetone, chloroform, dichloromethane, ethanol, methanol, isopropanol, *n*-hexane, acetonitrile, tetrahydrofuran (THF) and sulfuric acid (H_2SO_4) were obtained from Carlo Erba Reagents (Milan, Italy). The *n*-hexane and chloroform were distilled before their use. Furthermore, the calcium salt of fenoprofen (MW = 522.60 g/mol), Synperonic P105® (MW = 6500 g/mol), acryloyl chloride, triethylamine, sorbitan trioleate (Span 85), polyoxyethylene sorbitan (Tween 85), N,N,N′,N′-tetramethylethylenediamine (TMEDA), *N*,*N*′-methylene-bisacrylamide (MBA), ammonium persulfate (APS), dicyclohexylcarbodimide (DCC), dimethylaminopyridine (DMAP), sodium hydroxide, and 5-fluorouracil were purchased from Sigma-Aldrich (Sigma Chemical Co., St. Louis, MO, USA).

2.2. Measurements

FT-IR spectra were measured by a Jasco 4200 IR spectrophotometer using KBr disks in the range of 4000–400 cm^{-1} (number of scan 16). ^{1}H-MNR spectra were recorded on a Bruker VM-300 ACP NMR spectrometer; the chemical shifts were expressed as δ-values (ppm) and referred to the solvent. The UV–Vis spectra were carried out using a JASCO UV-530 spectrophotometer. The samples were lyophilized by using a freezing-drying equipment (Micro modulyo, Edwards). Scanning electron microscopy (SEM) analysis of microspheres was performed with a JEOL JSMT 300 A microscope; the surface of the samples was made conductive by deposition of a thin gold layer in a vacuum chamber. The liquid crystalline properties of compounds were investigated by a polarizing optical microscope, POM (Laborlux 12 POL, Leitz, equipped with a heating stage, PR 600, Linkam) and differential scanning calorimetry. The transition temperatures were evaluated with a differential scanning calorimeter (DSC-200, Netzsch and DSC-141, Setaram) operating under nitrogen atmosphere at a heating rate of 10 °C min^{-1}. The mass of samples (around 4.0 mg) was placed in aluminium crucibles with pierced lids. Liquid crystalline phases were identified through the comparison of the texture changes around the transition temperatures with reference textures [27].

2.3. Preparation of fenoprofen

The acid form of fenoprofen was obtained from the corresponding salt. Briefly, 1 g of fenoprofen calcium salt was solubilized in 400 ml of distilled water and heated to 100 °C under magnetic stirring (200 rpm) for 1 h. Then, H_2SO_4 was added to promote the precipitation of fenoprofen acid, which was recovered by filtration, through porous glass filters with porosity grade 4, dried with a mechanical vacuum pump and then characterized by FT-IR and ^{1}H NMR.

2.4. Esterification of poloxamer with fenoprofen

In a three-neck flask fitted with a reflux condenser, a dripping funnel and a magnetic stirrer, thoroughly flamed and maintained under nitrogen bubbling, 0.100 g of fenoprofen acid (0.412 mmol) were dissolved in 50 ml of dry chloroform. Then, DCC ($8.5 \cdot 10^{-2}$ g, 0.412 mmol) and DMAP ($2.5 \cdot 10^{-3}$ g, $2.06.10^{-2}$ mmol) were added and, after 15 min, poloxamer (1.34 g, 0.206 mmol) was dispersed in the reaction mixture. It was kept cold, in a cold-water bath, stirred for 12 h and continuously monitored by thin layer chromatography (TLC) (silica gel, eluent mixture chloroform/n-hexane 3/7). Finally, the solvent was removed at reduced pressure. The raw product was

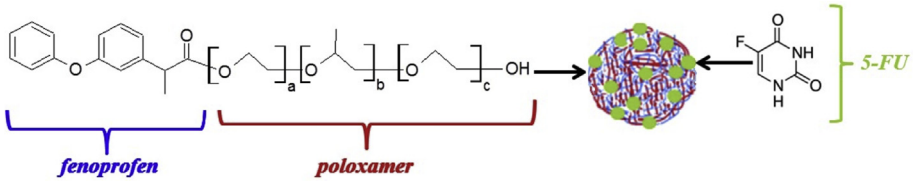

Fig. 1. Representation of microspheres based on fenoprofen and poloxamer.