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Pluronic/gelatin composites for controlled release of actives

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ABSTRACT

This paper describes the preparation and the release properties of composite materials based on Pluronic F127 and gelatin hydrogels, which could be of interest in the field of enteral nutrition or drug administration. The composites were prepared by exploiting the opposite responsivity to temperature of a 20% w/w Pluronic F127 aqueous solution (critical gelation temperature around 23 °C) and gelatin (gel–sol temperature transition around 30 °C). Pluronic domains dispersed within a gelatin matrix were obtained by injecting cold Pluronic F127 solutions inside hot gelatin solutions, while homogenizing either with a magnetic stirrer or a high-energy mechanical disperser. Calorimetry indicates that the composites retain the individual gelling properties of Pluronic and gelatin. Different releasing properties were obtained as a function of the preparation protocol, the temperature and the pH. The release profiles have been studied by a Weibull analysis that clearly points out the dominating role of gelatin at 25 °C. At 37 °C the release accounts for a combined effect from both Pluronic F127 and gelatin, showing a more sustained profile with respect to gelatin hydrogels. This behavior, together with the ability of Pluronic F127 to upload both hydrophilic and hydrophobic drugs and flavors, makes these innovative composite materials very good candidates as FDA-approved carriers for enteral administration.

1. Introduction

Hydrogels are three-dimensional polymeric networks capable of uploading high amounts of water or biological fluids [1], commonly used in a wide range of applications such as cosmetic, pharmaceutical, biomedical and food industry [2].

In the past few years, hydrogels have been extensively studied in the development of smart drug delivery systems [3]. As a matter of fact, these systems offer several advantages that potentially improve the pharmacological and therapeutic properties of the administered drugs. For instance, hydrogels can protect the active agents from hostile environments (e.g., the low pH in stomach or the presence of enzymes), as well as to control the release in response to environmental *stimuli*.

In the view of their applications in enteral nutrition or drug administration (*i.e.*, oral, sublingual and rectal administration of drugs and/or nutrients), hydrogels based on naturally-occurring polymers show optimal biocompatibility and allow for multiple strategies for drug delivery [4]. To this aim, gelatin is a very promising candidate, as it is a natural polymer obtained by the partial hydrolysis of collagen extracted from skin, bones, and connective tissues of animals [5,6]. Furthermore, composite materials made of gelatin hydrogels in combination with ceramics, natural and synthetic polymers have been developed for the controlled delivery of therapeutics and bioactive agents [7]. Composite systems combine two or more materials to generate a novel system with unique features, such as enhanced mechanical properties [8], responsivity to external *stimuli* [9,10], or controlled release [11,12]. In particular, it has been demonstrated that the release mechanism of therapeutic agents from gelatin-based composites can be tuned both via temperature [13] and pH [14]. However, their usage has been severely limited by high dissolution rate of the gelatin-based composites in physiological conditions [7], which has led to various cross-linking strategies to obtain a stable and biocompatible material. Unfortunately, cross-linking agents such as glutaraldehyde have been proven to be cytotoxic to different extents [15]. This has encouraged the research of new cross-linkers displaying reduced toxicity [16,17] and new approaches to improve their reactivity [18].

Pluronic F127 solutions are known to have a peculiar behavior with temperature: in particular above its critical micellization temperature (CMT) this polymer self-assembles into micelles and a further temperature increase leads to the interaction among micelles, eventually forming a hydrogel [19]. The process is fully reversible and the sol–gel transition temperature, indicated as criti-

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cal gelation temperature (CGT), can be easily tuned by changing the polymer concentration and the solvent [20,21]. This thermal behavior is thus opposite to that of gelatin, which around 30 °C turns from the gel state into a liquid [22]. For this reason the inclusion of Pluronic F127 into a gelatin matrix could be used to formulate a composite with significantly reduced dissolution rate in physiological conditions. In addition, the co-formulation of gelatin with Pluronic F127 does not introduce any toxicity concern, as Pluronic F127 is already included in both GRAS (Generally Recognized As Safe) and IIG (Inactive Ingredients) lists from FDA (Food and Drug Administration) [23], being the reason for its wide used in commercial products, such as mouthwashes or contact lens cleaning formulations.

In this paper we describe a facile preparation method to obtain a novel composite material made of Pluronic F127 and gelatin. This Pluronic F127-gelatin composite (PGC) could be of interest in the field of enteral administration, both for oral drug administrations as well as for the food industry. In addition, the chemical nature of these materials enables the incorporation of either hydrophilic or hydrophobic molecules, as extensively reported in the literature [24], with tunable release kinetics. Aiming at the evaluation of the release properties of the PGCs, we selected the commercially available food dye azorubine (E122) as a model molecule. The composite was prepared following two routes that take advantage of the gelation of a Pluronic F127 water solution when injected into a hot gelatin solution. Two homogenization methods were investigated (magnetic stirring and high-energy Ultra-Turrax mixing), resulting in a composite gel and foam, respectively. In particular, the preparation of hydrogels and foams with the same composition allows for studying the effect of the interface area extension on the release properties. Foams have been recently reported as effective vehicles for the sustained delivery of drugs [25].

The release was studied as a function of pH and temperature to mimic the environmental conditions eventually experienced by the composite either in the oral cavity or in the stomach. Results clearly show that the proposed approach was successful in modulating the release mechanism. Furthermore, the presence of Pluronic hydrogel domains at 37 °C mitigates the effect of temperature on gelatin.

2. Material and Methods

2.1. Materials

Pluronic F127 (M_w = 12600 g/mol, Sigma–Aldrich, Milan, Italy), gelatin (from porcine skin, Fluka, Milan, Italy) were used as received without any further purification. Azorubine (E122, food grade quality) was supplied by F.lli Rebecchi S.r.l., Piacenza, Italy. Water used throughout this work was MilliQ grade (18.2 MΩ cm at 25 °C).

2.2. Samples preparation

20% w/w Pluronic F127 water solution was prepared by adding a weighed amount of polymer to ice-cold water under constant stirring [26]. The dispersion was then placed in a refrigerator overnight to obtain a clear solution. The hydrogel was obtained by warming up the solution to room temperature, RT. Azorubine-loaded Pluronic F127 hydrogels for release experiments were prepared following the aforementioned procedure, using a 20 μg/mL azorubine aqueous solution instead of pure water. 20% w/w gelatin water solution was prepared by heating up at 50 °C under constant magnetic stirring until a pale yellow clear solution was obtained. The corresponding hydrogel was obtained by cooling down the solution to RT. Pluronic-gelatin composites (PGCs) were prepared according to two sequential procedures (see Fig. 1A). In the first case, a cold Pluronic F127 solution (0.3 mL) was injected by a syringe (needle = 27 G, nominal inner diameter = 0.150 in) in a mechanically stirred gelatin solution (2.5 mL) at 50 °C. The stirring was continued until a macroscopically homogeneous dispersion was obtained and cooled down to RT (hereinafter referred to as PGC-I). A second Pluronic F127-gelatin composite (PGC-II) was prepared similarly to PCG-I, except for the mechanical mixing that was performed by means of a ULTRA-TURRAX® (UT) disperser, equipped with a S 25 N head (IKA- Werke GmbH & Co., Staufen, Germany) working at 9.500 rpm for 5 min, and 24.000 rpm for 10 additional minutes. The resulting material consists in a composite foam.

2.3. Release experiments

The released amount of dye as a function of time from azorubine-loaded PGC-I and PGC-II dispersions was evaluated by UV–visible spectroscopy with a Cary 100 Bio. 20 spectra per sample were acquired in the 400–700 nm range. In order to avoid sample interference with the beam path, weighted amounts of either gelatin hydrogel or PGCs were supported into the optical cuvettes (see Fig. S1 in the supplementary material) with the aid of metallic grids (standard U.S. mesh size = 10) and put into contact with artificial saliva (modified version of Poggio et al. [27], details are given in Table S1 of the supplementary file) at different pH values (2 and 7) and different temperatures (25 °C and 37 °C).

2.4. Field emission scanning electron microscope (FE-SEM)

FE-SEM images were collected using a ΣIGMA (Carl Zeiss Microscopy GmbH, Germany) operating at 1 kV on uncoated samples. All samples were freeze-dried for 24 h prior the measurements using a BenchTop 2K freeze-dryer (VirTis, USA) under 30 mTorr vacuum at −50 °C.

2.5. Differential scanning calorimetry (DSC)

DSC measurements were obtained by means of a Q2000 (TA Instruments, Philadelphia, USA) on ∼15 mg samples in aluminum hermetic pans, with a temperature program from −5 °C to 70 °C at 5 °C/min. Analysis of DSC endotherm peaks was performed by means of Igor Pro 6.36, using an exponentially modified Gaussian (EMG) function:

$$f(T) = \sqrt{\frac{\pi}{2}}\frac{hw}{|s|}\exp\left(\frac{T_0 - T}{s} + \frac{1}{2}\left(\frac{w}{s}\right)^2\right)\cdot \mathrm{erf}\left(\frac{\frac{1}{2}(T_0 - T)}{w + \frac{w}{|s|}}\right) \quad (1)$$

where h is the height, T_0 the center, w the width of the peak and s is the distortion factor (shape).

For the sample referred to as *unmixed*, a piece of gelatin was placed inside the pan next to a piece Pluronic hydrogel (both at 25 °C and in the same relative amount as in the composites). This sample was prepared to verify that the thermal features of both gelatin and Pluronic F127 hydrogels could be detected for binary systems.

2.6. Thermogravimetric analysis (TGA)

TGA was performed by means of a SDT Q600 (TA Instruments, Philadelphia, USA), with a linear temperature ramp from 25 °C to 450 °C, at a rate of 10 °C/min and a constant nitrogen flow rate of 100 mL/min.

3. Results and discussion

Fig. 1 reports a scheme of the PGCs preparation protocols (Fig. 1A), as well as their visual aspect (Fig. 1B). Compared to gelatin aqueous solution, PGC-I displays a slightly hazy aspect (Fig. 1B top),