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Miscibility, interactions and antimicrobial activity of poly(ε-caprolactone)/chloramphenicol blends

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ABSTRACT

Poly(ε-caprolactone) (PCL) has been blended with Chloramphenicol (CAM), a well-known bacteriostatic antibiotic, in order to obtain new biomaterials with antibacterial properties. The resulting samples have been thoroughly characterized regarding both their physicochemical behavior and antimicrobial efficacy by means of very diverse techniques. Hence, PCL/CAM blend miscibility has been analyzed by Differential Scanning Calorimetry (DSC) using the single glass transition temperature (T_g) criterion, intermediate between those corresponding to the two components in the blend. In turn, the interaction parameter has been obtained from the analysis of the melting point depression in both PCL-rich and CAM-rich blends. Fourier-Transform Infra-Red (FTIR) spectroscopy and X-Ray Diffraction (XRD) analysis have been used -in the pure components and in the blends- to analyze both the specific interactions and the crystallization behavior, respectively. The morphology of PCL/CAM blends obtained by spin-coating has been also studied by means of Atomic Force Microscopy (AFM). Finally, drug release kinetics of different PCL/CAM systems as well as their antibacterial efficacy against *Escherichia Coli* have been investigated, indicating that CAM can be released from the PCL/CAM blends in a controlled way while keeping intact the antibacterial efficiency.

1. Introduction

A controlled drug-delivery device is that able to deliver the drug at the desired release rate and duration, thus maintaining the drug level in the body within the therapeutic window (see Scheme 1). Upon intake, drug molecules must be dissolved in aqueous-based gastrointestinal fluids in a sufficient quantity as to reach their whatsoever therapeutic effect. Nevertheless, most drugs are in crystalline form, which implies auto-association interactions and low water solubility [1]. A successful strategy to improve the solubility of poorly soluble drugs is to dispense the drug in the form of amorphous solid dispersions (ASDs).

The dispersion of a drug into a polymeric matrix enables the establishment of strong specific interactions with its functional groups, provided that they have complementary interacting groups. Thus, the chemical potential of the drug in the amorphous phase is reduced, and as a result, its crystallization can be hindered. In other words, miscibility between the two components should favor the formation of ASDs, preventing the crystallization of the drug, and as a result, enhancing its solubility [2]. The drug release mechanism from a polymer depends on parameters such as the nature of the polymer, matrix geometry, drug-related properties, initial drug loading, and drug-matrix interactions [3]. If, in addition, a medical device is made of a biodegradable polymer, it does not have to be removed after finishing its task. Furthermore, in a biodegradable polymer-drug system the control of the degradation rate of the biodegradable polymer enables the control of the drug release from the matrix [4].

In this regard, poly(ε-caprolactone) (PCL) is one of the most promising biodegradable polymers and is already widely used in biomedical applications. PCL is suitable for long-term biomedical applications since its degradation can last from several months to years [5]. In turn, Chloramphenicol (CAM) is a broad-spectrum bacteriostatic antibiotic, which diffuses through the bacterial cell and binds to 50S ribosomal subunit. Such interaction induces the inhibition of the bacterial protein synthesis as well as the blocking of bacterial cell proliferation. A wide range of microorganisms can be effectively treated by CAM [6]; for example, it is useful in the treatment of staphylococcal brain abscesses

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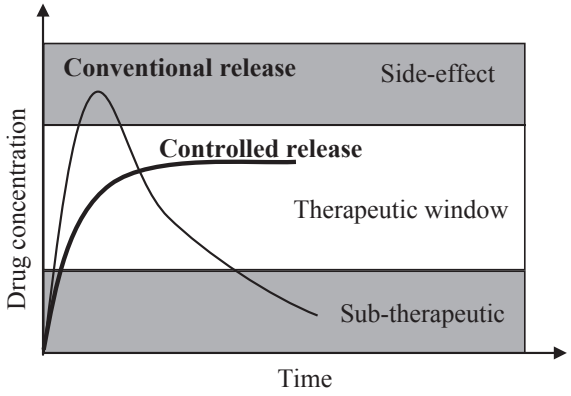


Scheme 1. A comparison of conventional drug delivery profile vs. a controlled drug release profile.

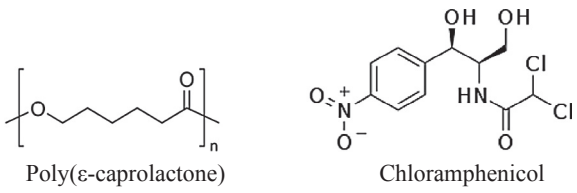
due to an excellent blood-barrier penetration or meningitis caused by *Enterococcus faecium*. Its employment has also been shown to be effective in treating ocular infections caused by a number of bacteria including *Staphylococcus aureus*, *Streptococcus pneumoniae* and *Escherichia coli* [7]. As main disadvantage CAM shows low aqueous solubility [8].

Considering the respective chemical structures of the two species under study (see Scheme 2), hydrogen bonding can be expected to be formed between the hydroxyl groups of CAM and the carbonyl groups of PCL. In this paper, the miscibility of the PCL/CAM blends is analyzed using the criterion of the single intermediate glass transition temperature (T_g), while the strength of the resulting intermolecular interactions is evaluated from the interaction parameter. FTIR spectroscopy is then used to analyze the specific interactions responsible for the miscibility of the blends. Moreover, morphological changes in the blends are discussed in terms of PCL/CAM intermolecular interactions through the analysis of the spherulitic growth by means of AFM. The suitability of the blends for biomedical applications has been analyzed by investigating the release kinetics of CAM in PBS solution at 37 °C, as well as by analyzing the antibacterial efficacy of the PCL/CAM blends against *Escherichia Coli* conducting the agar diffusion test. *E. coli* is a gram-negative etiologic agent associated with biofilm formation on implants and susceptible to chloramphenicol treatment. The results obtained by these techniques show that CAM can be released in a controlled way from the PCL/CAM blends.

2. Experimental section

2.1. Starting materials

Poly(ε-caprolactone) (PURASORB® PC12 trade name) with an average molecular weight (M_w) of $1.3 \cdot 10^5$ g mol^{-1} and $M_w/M_n = 1.76$ (as determined by GPC) was obtained from Purac Biochem (The Netherlands). Chloramphenicol (purity ≥ 98%) and Phosphate Buffered Saline (PBS) solution 1 M (pH 7.4) were supplied by Aldrich Chemical Cor (Spain). *Escherichia coli* (ATCC 1655) was purchased from ATCC (Virginia, USA).



Scheme 2. Chemical structures of PCL and CAM.

2.2. Blend preparation

Films were prepared by casting from tetrahydrofuran (THF) solutions at room temperature. Films 100 μm thick were prepared by casting from 2.7 wt% solutions into 60 mm diameter Petri dishes, and films 800 nm thick were obtained by spin-coating onto glass substrates from 10 mg/mL solutions using a spin-coater (Schaefer Tech) operating at 1000 rpm for 60 s.

2.3. Differential Scanning Calorimetry (DSC)

Thermal analyses were conducted on a Modulated DSC Q200 from TA Instruments. All the scans were carried out in hermetic aluminum pans under nitrogen atmosphere with sample weights between 5 and 10 mg. In order to study the glass transition temperatures, two consecutive scans were performed from −80 °C to 180 °C with a scan rate of 20 °C/min, ensuring complete melting of the sample. Glass transition temperatures (T_g) were measured in the second scan as the midpoint of the specific heat increment.

2.4. Melting point depression

The melting point depression of CAM was investigated from CAM-rich blends containing 100–70 wt% CAM. Samples were heated in the DSC with a scan rate of 1 °C/min to obtain the melting temperature of the CAM crystals. No weight loss was observed during the thermal treatments.

The melting point depression of PCL was analyzed from PCL/CAM blends containing 100–80 wt% PCL. Samples were first heated at 180 °C for 3 min to assure complete melting of the PCL and CAM crystals, and then cooled at 10 °C/min to the desired crystallization temperatures (T_c) allowing to crystallize isothermally for 60 min. Then, they were heated to 180 °C to obtain the melting points with a scan rate of 10 °C/min.

2.5. Fourier transform infrared spectroscopy (FTIR)

FTIR spectra were recorded on a Nicolet AVATAR 370 Fourier transform infrared spectrophotometer. Spectra were taken with a resolution of 2 cm^{-1} and were averaged over 64 scans in the 4000–450 cm^{-1} range. Tetrahydrofuran solutions (1.1 wt%) were cast on KBr pellets by evaporation of the solvent at room temperature. Traces of tetrahydrofuran were removed placing the films into a heated vacuum oven for 24 h. The absorbance of the samples was within the range where the Lambert-Beer law is obeyed. A controlled high temperature transmission cell mounted in the spectrometer was used to obtain the spectra of molten samples. Second derivative spectra were smoothed using the Norris-Williams Gap Derivatives [9] using maximum gap sizes and segment lengths of 5 points and 5 cm^{-1} respectively in the derivative transformations.

2.6. X-ray diffraction (XRD)

X-ray powder diffraction patterns were collected by using a Philips X'pert PRO automatic diffractometer operating at 40 kV and 40 mA, in theta-theta configuration, secondary monochromator with Cu-Kα radiation (λ = 1.5418 Å) and a PIXcel solid state detector (active length in 2θ 3.347°). Data were collected from 5 to 50° 2θ (step size 0.026 and time per step = 700 s) at room temperature. A fixed divergence and antiscattering slit giving a constant volume of sample illumination was used.

2.7. Atomic force spectroscopy (AFM)

An AFM instrument Nanowizard I (JPK Instruments, Germany) was operated in air, using contact imaging mode at constant loading forces