NANOPARTICLES BASED ON STAR-SHAPED CARBOXY-TERMINATED POLYLACTIDE AND CHITOSAN FOR CONTROLLED RELEASE APPLICATIONS

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Abstract

In this work chitosan based nanoparticles were prepared and modified by a star-shaped carboxy-terminated polylactide (S-PLA) in order to reduce the doxorubicin burst effect in physiological solution.

S-PLA was prepared by polycondensation reaction using pentetic acid as a core molecule and methansulfonic acid as catalyst and linked to chitosan backbone through coupling reaction. Afterwards, chitosan-(S-PLA) modified nanoparticles loaded with doxorubicin were prepared by polyelectrolytes complexation method using dextran sulfate sodium salt as polyanion.

Results show nanoparticles with diameter in the range 100-200 nm, spherical shape and ζ-potential between 27-35 mV. The presence of (S-PLA) side chain does not influence the encapsulation efficiency of the drug but strongly reduce the initial burst effect compared with unmodified chitosan.

Keywords: burst effect, drug delivery, chitosan, polylactide, doxorubicin

1. INTRODUCTION

The controlled release of bioactive molecules from polymeric nanoparticles has attracted the attention of many researches in the last years [1]. Different controlled release systems have been prepared to obtain predesigned release profiles. In many formulations an initial large amount of drug is released immediately upon placement in the release medium [2]. This phenomena known as “burst effect “ not only cause an initial high level of drug delivery but also reduce the lifetime of the device. One of the most difficulties related to the burst is that the intensity is hardly predictable. In some cases burst effect could be a goal, in particular in pulsatile delivery where loaded molecules can be released rapidly upon changes in environmental conditions such pH, temperature or ionic strength [3]. Different physical, chemical or processing parameters regarding the carrier and the loaded drug can cause the initial burst. To prevent it several technologies are available like surface extractions, coating or modification, but all of them involves additional costly steps which also results in reduced drug loading or introduction of additional materials [3].

In this work doxorubicin (DOX) was chosen as model drug as is widely used in anticancer therapy. In order to reduce the initial burst, chitosan (CS) was modified with star-shaped carboxy-terminated polylactide (S-PLA). S-PLA was prepared by polycondensation reaction using pentetic acid as a core molecule, methansulfonic acid as catalyst and subsequently linked to CS using EDC as activator [4]. Nanoparticles were prepared by complexation method using dextran sulfate (DS) as polyanion (CS / DS weight ratio: 2) and characterized in terms of dimension and ζ-potential by dynamic light scattering (DLS) and morphology by transmission electron microscopy (TEM) . The effect of the S-PLA side chain on DOX encapsulation and release in physiological solution at 37 ˚C was analyzed and compared with unmodified CS.

2. MATERIALS AND METHODS

2.1 Materials

Low molecular weight chitosan (D.D 75-85%); N-hydroxy-succinimide; doxorubicin hydrochloride 98.0-102.0%, N-(3-dimethylaminopropyl)-N’-ethylcarbodiimide hydrochloride, commercial grade, N,N-

diethylformamide 99%, dimethyl sulfoxide in deuterated form (DMSO-d6), pentetic acid (PA) (*N,N*-Bis(2-bis(carboxymethyl)amino]ethyl)glycine, ≥99.5%), methanesulfonic acid (MSA, ≥95%) and hydrochloric acid (HCl, 30% for trace analysis) supplied by Sigma Aldrich. The solvents acetone, ethanol, methanol, indicator phenolphthalein, potassium hydroxide, (all analytical grade)were bought from IPL Lukes, Uhresky Brod, Czech Republic. Chloroform $CHCl_3$ (HPLC grade) was purchased Chromspec, Brno, Czech Republic. L-lactic acid (L-LA, 80% water solution), was purchased from Merci s.r.o., Czech Republic, Tetrahydrofuran (HPLC grade) was purchased from Chromservis, Czech Republic. All chemicals were used as obtained without further purification.

2.2. Star shaped carboxy-terminated PLA preparation, characterization and grafted to CS

S-PLA was preparing according with the polycondensation reaction procedure reporting in a previous paper [4]. The concentration of terminal –COOH groups was determined by titration in dichloromethane with 0.01 M KOH ethanol solution using phenolphthalein as indicator. The –COOH concentration was obtained according with Eq. 1 :

$$C_{COOH} = \left(\frac{(a-b)* N}{m} \right) \quad [1]$$

Where *a* and *b* are the volume (ml) for titration of sample and blank, respectively, *N* the normality of KOH (mol/l) and *m* the sample weight (g).

Molecular weight of the product was characterized by GPC (Agilent HT-GPC 220) equipped with a dual detection system (refractive index and viscometric detector).

The copolymer (CS-(S-PLA)) was prepared according with the procedure reported in published work [5].

2.4 Doxorubicin loaded CS and CS-(S-PLA) nanoparticles preparation, characterization and release studies

Solutions of CS-(S-PLA) and CS (3 mg/ml) in acetic acid aqueous solution (1% v/v) were separately prepared. DOX (1 mg/ml; V=1 ml) was dissolved in water and added to 1mg/ml aqueous solution containing DS and stirred for 3h. Afterwards, the resulting solution was added dropwise to CS and CS-(S-PLA) under vigorous stirring.

Dynamic light scattering (Nano ZS Malvern) and TEM (JEOL 2O10F) analysis were used to investigate the dimension, ζ-potential and morphology of the nanoparticles in preparation media.

Encapsulation efficiency (*EE*) of DOX was determined via UV-Vis spectrophotometer (Cary 300 Varian) at 480 nm. The amount of drug was calculated from a calibration curve, prepared by measuring the absorbance intensity of the known drug concentration. *EE* was calculated as follows:

$$EE = \left(\frac{D_t - D_f}{D_t} \right) \times 100 \quad (2)$$

D_t = Total amount of drug (mg/ml); D_f = Amount of drug untrapped (mg/ml).

The effect of S-PLA side chain on the release rate of DOX was evaluated and compared with unmodified CS in physiological solution at 37° C. 10 mg of each samples was suspended in 10 ml of media and at predetermined time intervals, 1ml was withdrawn and replaced with fresh buffer to maintain the total volume. The percentage of drug released (*DR*) was detected by UV-Vis spectrophotometer at 480 nm and calculated by the following equation

$$DR = \left(\frac{D_t}{D_0} \right) \times 100 \quad (3)$$

Where D_t represents the amount of drug released at a time t (mg) and D_0 the amount of drug loaded (mg). All studies were done in triplicate.

3. RESULTS

3.1 S-PLA and CS-(S-PLA) characterization

In Tab.1 the values of C_{COOH} titration and molecular weight of PLA and S-PLA are reported.

	C_{OH} (mmol/g)	C_{COOH} (mmol/g)	C_{COOH}/C_{OH}	M_n (g/mol)	M_w (g/mol)	D
PLA	0.346	0.346	1	2900	4700	1.6
S-PLA	0.284	0.979	3.45	1900	4000	2.4

Table 1 Characterization of PLA and S-PLA in terms of molecular weight and concentration of carboxylic groups

The values reported in Tab.1 show S-PLA C_{COOH}/C_{OH} ratio >1 due to the higher number of –COOH groups compared to PLA. Molecular weight (M_w) of both products is comparable while a higher polydispersity (D) is presented in S-PLA due the reaction with PA.

CS-(S-PLA) was characterized by FTIR-ATR analysis (NICOLET 320 FTIR, ZnSe crystal, 64 scan and 4 cm^{-1} resolution). The spectra shown two distintive features which confirmed that reaction occurered; a new peak at 1636 cm^{-1} ascribed to amide bond between CS and S-PLA and a more intense at 1407 cm^{-1} (C-N stretching).

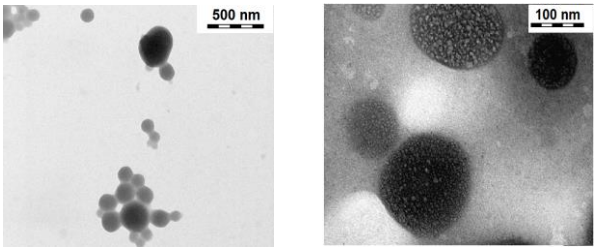
3.2 Nanoparticles characterization

In Table 2 dimension and ζ-potential of loaded and unloaded CS and CS-(S-PLA) nanoparticles are reported.

	Diameter (nm)	ζ-potential (mV)
CS	130 ± 32	+35 ± 0.9
CS-DOX	151 ± 26	+31 ± 0.5
CS-(S-PLA)	162 ± 43	+27 ± 0.7
CS-(S-PLA)-DOX	176 ± 31	+22 ± 0.3

Table 2 Diameter and ζ-potential of CS and CS-(S-PLA) based nanoparticles loaded and unloaded with DOX in preparation media

The nanoparticles diameter was found in the range 100-200 nm (Tab.2). Both systems showed a slight increase in diameter when drug was encapsulated. ζ-potential of all formulations was in the range 23-35 mV (Tab.1). The presence of drug slightly decreased the ζ-potential value, probably through a shielding effect.



a b

Fig. 1 TEM micrographs of unloaded a) CS and b) CS-(S-PLA) nanoparticles in preparation media

TEM micrographs reported in Fig.1 show nanoparticles in solution with spherical shape and correspondence with values for diameter reported in Tab.2.

3.2 DOX encapsulation and release studies

DOX was loaded into nanoparticles during the preparation. Generally, the drug carrying capacity of the nanoparticles is defined in terms of *EE*. The *EE* in this systems is strictly correlated with the pH of the medium, as previously demostrated [6]. In this case pH 5.5 represents an optimal value for CS and CS-(S-PLA) dissolution and surface charge. Both systems, CS and CS-(S-PLA) demonstrated *EE* of 85% and 80%, respectively, meaning that around 200 μg of DOX per mg of carrier was loaded. It indicates that S-PLA side chain slightly influence the loading with a decrease in *EE* of only 5%. However, the *EE* is high and fully comparable to other polymeric and not nanoparticles systems reported in literature [6-8].

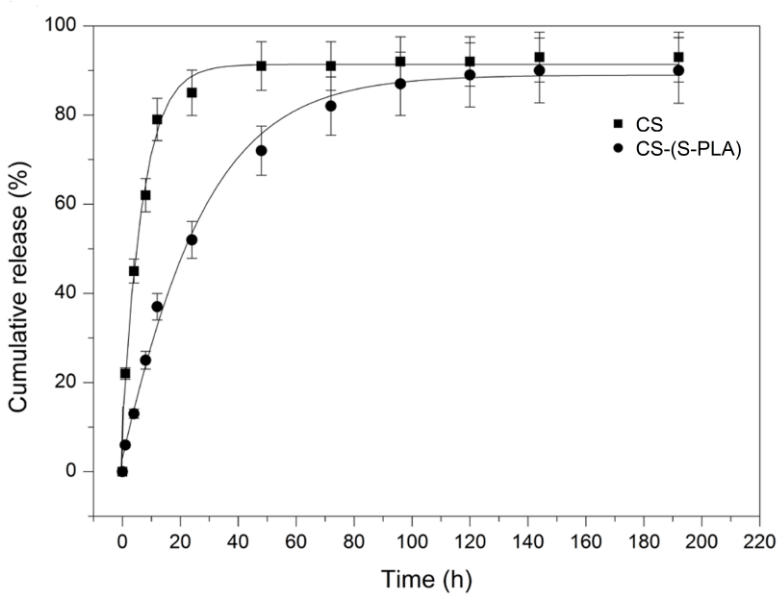


Fig.2 Release kinetic trend of DOX from CS and CS-(S-PLA) in physiological solution at 37 ˚C.

In Fig.2 DOX release profiles from CS and CS-(S-PLA) are reported. As can been seen in almost two days up to 80 % of the drug was released from both systems while almost 90% in 50 h in case of CS and in 120 h for CS-(S-PLA) As DOX release from these systems occurs by diffusion following swelling, the presence of the hydrophobic chains could retard the medium intake in the core system slowing the diffusion of the drug towards the surface and consequently prolonging the release time. The main effect of S-PLA side chain was more enhanced in the first 0.5 h and 1 h after contact with the media where the intensity of burst effect was reduced up to 30% compared with unmodified CS.

CONCLUSIONS

In this study, nanoparticles made by grafting star-shaped carboxy-terminated PLA to CS backbone were prepared. S-PLA was synthetized by polycondensation and subsequently linked to CS through coupling reaction between amino group of CS and carboxylic group of PLA. Nanoparticles were obtained by complexation technique using DS as polyanion. The average size of CS and CS-(S-PLA) nanoparticles felt in the range 100-200 nm and the ζ-potential between 27-35 mV suggesting good stability in preparation media. The presence of PLA side chains do not affect the encapsulation efficiency of DOX, which is between 80-85% corresponding to 200 μg per mg of carrier. *In vitro* release studies showed a sustained and controlled release of DOX where up to 90% released in 2 days in CS and in almost 5 days in case of CS-(S-PLA) Additionally, release profile demonstrated significant reduction of the burst effect in the first 30 minutes in presence of S-PLA and a reduction of the release rate in the first 12 h more than 50% compared to unmodified CS.

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