

Drug delivery system based on polymer nanofibers

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Abstract

This work presents our attempts to characterize release of two model drugs from electrospun polymer nanofibers. Such drug delivery system offers great potential for applications in medicine especially as neurosurgery protective membranes. Proper delivery of drugs requires precise control of the drug diffusion process during the release for days or even weeks. Lipophilic model drug Rhodamine B and hydrophilic Bovine Serum Albumin conjugated with Fluoresceine (BSA-FITC) were embedded in electrospun poly(L-lactide-co- ϵ -caprolactone) (PLC) nanofibers. Release of Rhodamine B showed saturation in cumulative release profile at 60% and 86% for 1.5% and 3% wt. initial drug content, respectively. Nanofibers electrospun from emulsion released almost entire drug encapsulated in water vesicles inside the nanofibers. Possible location of vesicles close to the surface of the nanofibers exposed them for surrounding fluid and caused leaching of the drug. In this case encapsulation of drug in emulsion prevented the initial burst release. Dependence of a drug release and composition of nanofiber is essential for production of drug delivery systems. Mathematical model constructed with this data allows to avoid tedious experimental work.

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Introduction

The earliest reports considering interaction of strong electric field with stream of fluid can be found in the work of scientists of the seventeenth century when first attempts to understand the electrostatic phenomena began [1]. Reports from the early twentieth century on the nanofibers formation are mainly patents claiming the electrospinning process and commercialisation for the fabrication of textile yarns [2]. About twenty years ago, nanofibers reached a lot of interest due to possible application in medicine. Nanofibrous mats can be used as a wound dressings [3], drug delivery systems [4] or temporary scaffolds for cells culturing [5]. Electrospinning of lipophilic drugs dissolved in polymer solution results in fine nanofibers of diameter range of 50 – 500 nm with homogenously distributed drug inside them. A large number of hydrophilic drugs like proteins and growth factors can be electrospun with water in oil emulsion or core-shell method [6]. In emulsion electrospinning droplets of drug solution form the inner structure of the fiber while in latter method drug solution forms continuous core surrounded by polymer (shell) membrane. Lipophilic drugs, like tocopherol acetate were successfully electrospun for transdermal delivery systems [7]. Vitamin loaded cellulose acetate fibers of the average diameter of 247 nm were compared with casted films from the same materials. The nanofibrous materials gradually released entire dose of drug in 24 hours while vitamin-loaded films showed 50% burst release within 20 minutes without further release. Influence of drug form in polymer solution was investigated on popular anticancer drug: lipophilic doxorubicin base and hydrophilic doxorubicin hydrochloride [8]. Lipophilic drug was loaded onto a polymer structure resulting in fiber of an average diameter of 360nm. This system gave 20% release for first hour of experiment followed by no release for subsequent 6 hours. This release profile was attained to fast release of the drug from crystals on the nanofiber surface while the rest of the drug remained unreleased. Hydrophilic drug electrospun from water in oil emulsion gave average fiber diameters of 800nm. Drug release experiment gave no burst and gradual release to 100% during 6 hours. The release profile was explained by diffusion of drugs from droplets of water phase being close to the fiber surface.

In this paper we present release pattern of two fluorescent model drugs: lipophilic Rhodamine B and hydrophilic Bovine Serum Albumin conjugated with Fluoresceine (BSA-FITC). Both compounds are used for optimisation procedure of nanofibrous materials produced in our laboratory. Subsequently, materials were used as a neurosurgery protective membranes in animal model [9].

Materials and methods

2.1. Nanofibrous mats preparation

Polymer solution was prepared by dissolving 1g of poly(L-lactide-co- ϵ -caprolactone) (PLC, containing 70% L-lactide and 30% caprolactone units, Purac, Netherlands) in mixture of dimethylformamide (DMF, POCh, Poland) 1g and chloroform (CHCl₃, POCh, Poland) 9g. The electrospinning solution containing lipophilic model drug Rhodamine B (Sigma Aldrich) was prepared by dissolving 4 mg of fluorescent dye in 1.5 g of polymer solution. 4mg of hydrophilic Bovine Serum Albumin conjugated fluorescein isothiocyanate (BSA-FITC, Sigma Aldrich) was first dissolved in 50 mg of distilled water and added drop-wise to 1.4 g of polymer and 20mg of SPAN80 solution. Prepared mixture was agitated for 30 minutes by vortex to prepare stable water in oil emulsion. The electrospinning of both solutions was attained in self build electrospinning unit with flow rate of polymer solution at 800 μ l/h, and the operating voltage of 15 kV. The spinning distance between blunted needle (26G) and grounded rotating drum (1800 rpm) was 15 cm. The obtained nanofibers formed random pattern as it is shown in Fig. 3.1. The temperature and relative humidity in the electrospinning chamber were T = 22°C and 35%, respectively.

2.2. Drug release

Nanofibrous mats were cut to 1cm x 1cm pieces and weighted to define total mass of model drugs present in the material and then placed in vials filled with 1 ml of 0.01M phosphate buffer saline (PBS) solution. The aim of maintaining material in 37°C of PBS buffer was to imitate conditions of the human body. After selected time intervals, materials were placed in fresh PBS solution and resultant supernatants were frozen. All samples were protected from light to avoid photo bleaching. At the last day of release experiment samples were thawed and transferred to 96-wells plates (Carl Roth, Poland). For quantitative assessment of drug release, calibration curves of known concentration of model drugs were measured using spectrofluorometer (Fluoroscan Ascent, Thermo Scientific).

2.3. Morphology of the nanofibers

Scanning Electron Microscopy (SEM, Jeol, JSM 6390 LV, Japan) was used to characterize nanofibers morphology and material thickness. Before the SEM imaging specimens of fibers were mounted on a metal holder and coated with a gold using mini sputter coater (SC 7620, Polaron, United Kingdom) at accelerating voltage of 10 kV. Epi-fluorescence microscope (Nikon Eclipse E-50i) was used to examine model drug distribution in as-spun nanofibers and inside them. ImageJ (NIH, USA) module was used to calculate fibers diameter based on the SEM pictures. The procedure consists of measuring diameter of a hundred nanofibers.

Results and discussion

The average fiber diameters electrospun from PLC polymer and for two model drugs: Rhodamine B and BSA-FITC are presented in Table 3.1. Two initial concentrations of the drugs were used 1.5% and 3.0% (relative to the polymer). The nanofibers containing lipophilic drug Rhodamine B had smaller average fiber diameters than the two phase materials composed of hydrophilic drug BSA-FITC.

Table 3.1. The characteristic parameters of the prepared nanofibers samples containing model drugs: Rhodamine B and BSA-FITC.

MATERIAL	MODEL DRUG	MATERIAL WEIGHT (mg)	TOTAL DRUG CONTENT (μg) (%)	AVERAGE FIBERS DIAMETER (μm)	STANDARD DEVIATION (μm)
1	Rhodamine B	7.62	114 (1.5)	0.851	0.409
2	Rhodamine B	6.69	200 (3.0)	0.860	0.317
3	BSA-FITC	3.48	55 (1.5)	1.395	0.470
4	BSA-FITC	4.25	127 (3.0)	1.342	0.501

The morphology and fiber diameters distribution in nanofibrous mat containing lipophilic and hydrophilic model drugs is presented in Fig. 3.1. a) and b). Random fibers orientation observed in both cases shows that even for high values of cylindrical collector rotation (1800 rpm) fibers do not form aligned structures. Observed nanofibers did not form ribbons nor beads structures along their length.

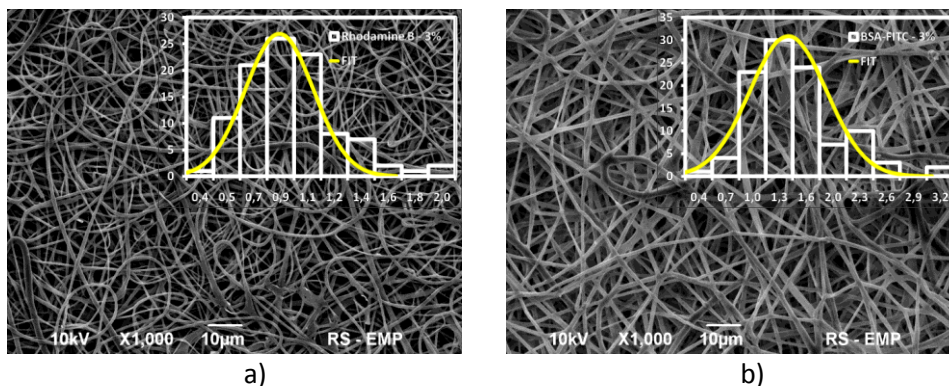


Figure 3.1. SEM micrographs and average fibers distribution in nanofibrous webs containing a) lipophilic Rhodamine B and b) hydrophilic BSA-FITC.

The model drug distribution was observed using fluorescence technique. The lipophilic Rhodamine B distribution (Fig.3.2a) is rather homogenous with higher fluorescence intensity observed at the intersections of the nanofibers. The electrospun suspension of vesicles with hydrophilic BSA-FITC formed fibers with visible clusters of fluorescent model drug (Fig.3.2b).

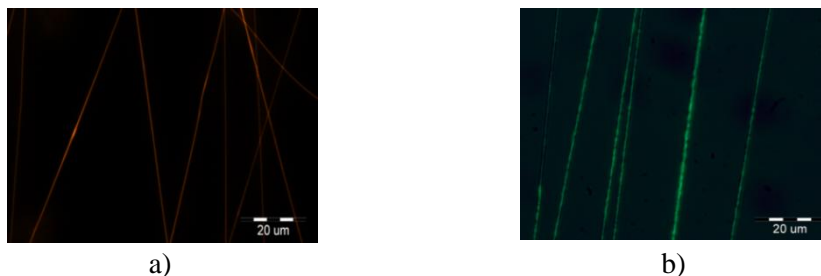


Figure 3.2. Fluorescence microscopy images presenting model drugs a) Rhodamine B and d) BSA-FITC distribution in the nanofibers.

The release profiles for two values of initial drug concentration are shown in Figure 3.3 and 3.4. The nanofibrous mat with 3% wt. lipophilic drug Rhodamine B (square points in Fig.3.3.a) showed 43% burst release in first hour and reached plateau at 86%. No burst release was observed for material with 1.5% wt. of Rhodamine B (diamond points in Fig.3.3.b) however material released only 60% of total drug present in the material during the course of release experiment.

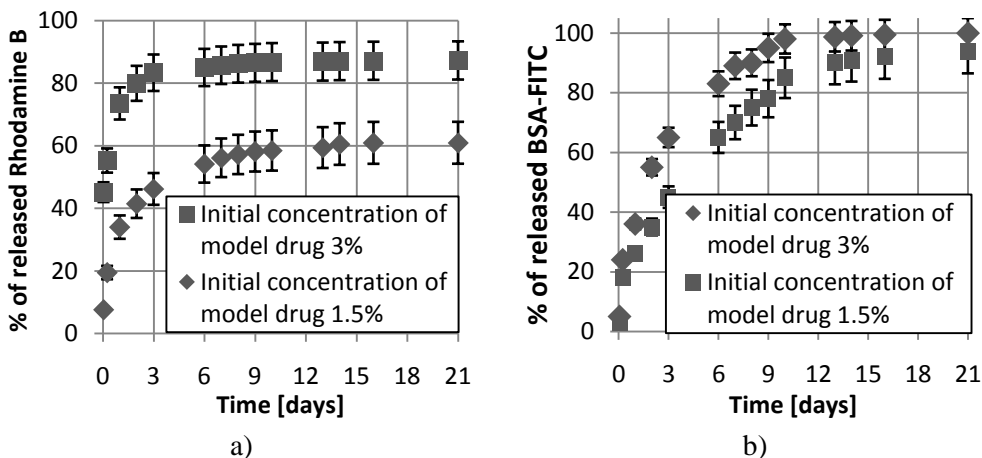


Figure 3.3. Cumulative release of a) lipophilic Rhodamine B b) hydrophilic BSA-FITC for two initial concentrations of model drug.

The materials electrospun from water in oil emulsion showed no burst release and on the contrary to Rhodamine B released almost entire drug to surrounding fluid. In case of 3% wt. (diamond points in Fig.3.3.b) BSA-FITC release rate was higher and release finished after 10 days. Material with smaller initial drug concentration – 1.5% wt. (square points in Fig.3.3.b), released drug during 21 days.

In case of Rhodamine B it is clear that part of the drug is captured in the bulk polymer and will be released during polymer degradation i.e. after about two years (according to manufacturer's statement). Small vesicles with BSA-FITC gradually released water-soluble compound without undesirable burst release. By changing other parameters in nanofibers composition e.g. polymer content, water to oil phase ratio one can adjust the rate of release to the required applications.

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