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Formulation design, preparation and characterization of multifunctional alginate stabilized nanodroplets

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ABSTRACT

In the present study the effect of process (homogenization speed) and formulation (polymer-alginate-concentration, surfactant concentration, drug amount, perfluorohexane volume fraction and co-surfactant inclusion) variables on particle size, entrapment efficiency, and drug release kinetics of doxorubicin-loaded alginate stabilized perfluorohexane nanodroplets were evaluated.

Particle size and doxorubicin entrapment efficiency were highly affected by formulation and process variables. Increase in homogenization speed resulted in significant decrease in particle size and increase in entrapment efficiency. Polymer concentration and perfluorohexane amount both had similar effect on particle size. Particle size increased by an increase in the amount of both. Entrapment efficiency increased by increasing polymer concentration. In case of surfactant concentration and drug amount, particle size and entrapment efficiency had optimum values and an increase in concentration of both of them behind a certain limit resulted in increase in particle size and decrease in doxorubicin entrapment.

In vitro release profile of doxorubicin was an apparently biphasic release process and 7%–13% of drug released after 24 h incubation in PBS, pH = 7.4, depending on the nanodroplets composition but ultrasound exposure for 10 min resulted in triggered release of 85.95% of doxorubicin from optimal formulation (formulation E1 with 39.2 nm diameter size and 92.2% entrapment efficiency).

1. Introduction

In the last decades, nanomedicine has been focused on the combination of various functionalities -e.g. therapeutic, imaging, and targeting function- in one molecular construct. These constructs may be macromolecules or nanoparticles. The family of important nanoparticles in biomedicine includes polymeric micelles, liposomes, hollow particles, nanoemulsions and nanodroplets.

Controlled drug delivery in combination with ultrasound has been extensively studied in the last decade [1–3]. Ultrasound is especially attractive because it is non-invasive, accessible, cost effective, and it is possible to combine imaging and therapeutic capabilities by ultrasound [4,5].

Liquid perfluorocarbon (PFC) nanodroplets are a new generation of ultrasound (US) contrast agents which combine the advantage of small size and ultrasound-aided release and imaging [4,6,7]. Low-boiling point perfluorocarbon nanodroplets, such as perfluorohexane (PFH) droplets, stay in their liquid form at 37 °C until exposed to ultrasound at sufficiently high rarefactional pressures [8], at which point the droplets vaporize and form gas bubbles. The selective acoustic activation of PFC nanodroplets makes them unique nanomedicine tools for theranostic – diagnostic imaging and therapy – application [9]. This droplet-to-bubble transition and bubble oscillation-or so-called cavitation- triggers release of encapsulated drug and enhances intracellular uptake [4,10,11]. Also, the bubbles respond non-linearly to ultrasound, which makes them suitable for using as ultrasound contrast agents for both B-mode and contrast-specific imaging techniques such as pulse-inversion [12]. Nanodroplets of hundreds of nanometers in size are able to extravasate in regions of tumor growth, while staying intravascular

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in healthy tissues [4], due to enhanced permeability and retention (EPR) effect of tumors [13].

Nanodroplets comprise a liquid-filled core and stabilizing shell of lipid, polymer and/or protein. Alginate was chosen for the stabilizing shell due to its favorable properties, including biocompatibility, low toxicity, relatively low cost and ease of gelation.

Alginate carriers have been used for variety of biological agents such as drugs, genes, and antigens to protect them during the transit in the human body [14,15]. Alginate gels can be used in controlled release of drug molecules, from small chemical drugs to macromolecular proteins depending on the cross-linker types and cross-linking methods. In addition, alginate gels can be orally administrated or injected into the body in a minimally invasive manner, which makes them ideal for pharmaceutical applications [16]. Alginate is a naturally occurring anionic polymer, extracted from marine brown algae. It is a water soluble salt of alginic acid consisting of a chain of (1–4)-linked β-D-mannuronic acid and α-L-guluronic acid in different arrangements of residues. Alginate is a biocompatible, biodegradable and muco-adhesive polymer [17].

There are several methods for synthesis of alginate particle such as gelation with calcium ions and other divalent cations [18] extrusion [19], emulsification [20] and ionic gelation [21]. Ionic gelation is one of the main methods of incorporating polymers to prepare hydrogels. The gelling property of alginate particles is affected by some factors such as physical condition, molecular weight and concentration of alginate polymer, cross-linking agent and type of surfactant [22].

Three important parameters that should be considered for preparing drug carries are the size of particles, drug entrapment efficiency and kinetic of drug release. The aim of the present study was to formulate doxorubicin loaded alginate stabilized PFH nanodroplets and evaluate the effects of formulation variables and process variables on particle size and entrapment efficiency of nanodroplets. Doxorubicin release kinetics, were also studied, for best samples selected from optimized formulations based on particle size to testify the potential application of these nanodroplets.

2. Materials and methods

2.1. Materials

Doxorubicin Hydrochloride (2 mg/ml) was obtained from EBEWE Pharma (Unterach, Austria). Sodium Alginate, Perfluorohexane and Tween 20 were supplied by Sigma-Aldrich (St. Louis, MO, Canada). Span 60 and Poloxamer 188 were purchased from Merck (Darmstadt, Germany). All other chemicals and solvents were of analytical grade and used as received without further purification or treatment.

2.2. Preparation of doxorubicin-loaded PFH/alginate nanodroplets

Doxorubicin-loaded nanodroplets were obtained via nanoemulsion process. Briefly, perfluorohexane (PFH), doxorubicin and Tween 20 (surfactant) were homogenized in distilled deionized water for 2 min at 1700 or 24,000 rpm using Ultra-Turrax SG215 homogenizer. Then, polymer solution (alginate 1.5%w/v) was added drop-wise whilst the mixture was homogenized at 13,000 rpm for 3 min. Finally, $cacl_2$ solution (0.2 w/v) were added dropwise to the emulsion under homogenization at 3000 rpm for 3 min. Nanodroplets were characterized for: morphology, by transmission electron microscopy (TEM); size distribution, and polydispersity index by dynamic light scattering; and drug entrapment and release by UV–vis spectroscopy.

The physicochemical properties of nanoparticles (e.g., particle size, entrapment efficiency and drug release kinetics) are affected by various process and formulation variables. To optimize the formulation of nanoparticles regarding size, drug entrapment and release kinetic, one process variable (homogenization speed) and different formulation variables (i.e., the amount of polymer, surfactant, drug, PFH and inclusion of a co-surfactant) were evaluated (Table 1).

2.3. Characterization of doxorubicin-loaded nanodroplets

All experiments were conducted in triplicate.

2.3.1. Particle size analysis

The average size distribution and polydispersity index of nanodroplets were determined by DLS using a Zeta-sizer 3000HS (Malvern Instruments, Malvern, UK). The analysis was performed at a scattering angle of 90° after the nanodroplet solution diluted adequately (1:1) with double-distilled water.

2.3.2. TEM

The morphology of drug-loaded nanodroplets was observed using a transmission electron microscope (H-7650; Hitachi, Tokyo, Japan) operating at an acceleration voltage of 80 kV. For sample preparation, one drop of the solution was placed onto a 400-mesh carbon-coated copper grid and dried at room temperature. It was then stained with 1% alkaline phosphotungstic acid (PTA) for several minutes and dried at room temperature before analysis.

2.3.3. Determination of entrapment efficiency

To determine the entrapment efficiency, drug-loaded nanodroplets were separated from the solution by centrifuging (centrifuges 5424 R, Eppendorf, Canada) at 11000 rpm for 30 min. Supernatants recovered from centrifuging were decanted. Doxorubicin content in the supernatant was analyzed by a UV–vis spectrophotometer at 480 nm (U.V-1601; Shimadzu, Japan). Samples were prepared and measured in triplicate.

Drug entrapment efficiency (EE) was calculated using the following equation:

$$\text{Entrapment Efficiency} = \frac{(\text{Total amount of drug added}) - (\text{Free amount of drug})}{\text{Total amount of drug added}} \times 100$$

2.3.4. In-vitro drug release study

2.3.4.1. Passive drug release. The release of doxorubicin from doxorubicin-loaded PFH/alginate nanodroplets was evaluated using phosphate buffer (pH 7.4) at 37 °C. 2 ml of the nanodroplet solution was poured into a dialysis bag (Spectrapor, MW cutoff 12000 g/mol) and placed into 10 ml of phosphate buffer (pH 7.4) severally. The release study was carried out in a shaker incubator (MS MP8 Wise Stir Wertheim, Germany) with shaking rate of 100 rpm at 37 °C for 24 h. At predetermined time intervals (0–6 and 24) the sampling was performed. In each time point, 2 ml of the buffer was elicited, and then replaced with an equivalent volume of fresh buffer. The amount of released doxorubicin in the buffer was analyzed by a UV–vis spectrophotometer (U.V-1601; Shimadzu, Japan) at a wavelength of 480 nm.

The accumulated release was calculated utilizing the following equation:

$$R = \frac{[V\ \Sigma n - i(Ci + V0\ C)]}{mdrug}$$

where, R is the accumulated release (%), V is the sampling volume, V0 is the initial volume, Ci and Cn are the doxorubicin concentrations, i and n are the sampling times, and mdrug is the mass of doxorubicin in nanoparticles.