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Original Research Paper

Stability of freeze-dried pH-responsive dextrin nanogels containing doxorubicin

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ABSTRACT

Induction of non-specific toxicities by doxorubicin (DOX) has restricted conventional DOX-based chemotherapy. pH-responsive dextrin nanogels (DNGs) have been fabricated in order to incorporate and deliver DOX to specific (targeted) sites. However, adequate stability studies of DOX-loaded DNGs are required for selection of storage conditions. The aim of this study was therefore to evaluate the accelerated (25 °C/60% RH) and long-term (5 °C) stability of DNGs prepared with formaldehyde (FDNGs) and glyoxal (GDNGs) as cross-linker by determining the change in their physicochemical properties. The mean diameter decreased with time during long-term storage. The drug content between freshly prepared (initial day) and after storage at 5 °C for 180 days of DOX-loaded FDNGs and DOX-loaded GDNGs was not significantly different ($p > 0.05$), but decreased after storage under the accelerated condition. The release of DOX from all DNGs was pH-dependent. However, DNGs kept under the accelerated condition showed higher amount of DOX release than those stored at 5 °C and the freshly prepared ones. The results indicate that the stability of DNGs could be improved by their storage at 5 °C.

1. Introduction

Cancer is a major cause of mortality worldwide with 8.2 million people being affected in 2012 [1]. Major clinical treatments for cancer include surgery, radiation, and chemotherapy, with chemotherapy being the major form. However, chemotherapy is a major form of management of cancer patients enlisting the used drugs to kill cancer cells. Among such drugs, the anthraquinonedoxorubicin (DOX) is a frontline

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chemotherapeutic agent used for treatment of several forms of cancer. Its mechanism of action is to inhibit DNA polymerases and topoisomerases and block the cell cycle, usually resulting in the induction of apoptosis in tumor cells [2,3]. Despite its efficacy, the clinical use of unformulated (free) DOX is limited due to development of progressive cardiomyopathy with apoptosis induction in cardiomyocytes by activation of p53 protein and reactive oxygen species leading to congestive heart failure [4]. In addressing this problem, a variety of innovative approaches to entrap this drug in nanocarriers and hopefully achieve site-specific delivery has been developed.

Among these approaches, pH-responsive nanocarriers have been previously exploited for targeted delivery of drugs. Due to its biocompatibility and degradability [5], dextrin is frequently chosen for nanogels formulating in order to circumvent carrier toxicity. It is a saccharide-based polymer containing D-glucose units linked by α-(1→4) glycosidic bonds, and considerable quantities of hydroxyl groups that are readily modified. Regarding biomedical application, dextrin is employed as a drug delivery system [5–9] and as a scaffold material [10,11]. pH-responsive dextrin nanogels (DNGs) are cross-linked dextrin networks fabricated by incorporating pH-responsive bonds, namely acetal bonds, into their structure. These bonds are used as linkers to immobilize anti-tumor drugs within the carrier matrix. In this system, DNGs are delivered to the tumor site *via* the enhanced permeability and retention (EPR) phenomenon [12,13]. DNGs are stable at physiological pH but could be destabilized and release the drug under mild acidic conditions at the target neoplastic site, resulting in enhanced therapeutic efficacy and reduced side-effects to normal tissue. Despite DNGs providing many benefits, the challenge remains in producing highly stable forms of encapsulated DOX and maintaining the long-term pH-responsive behavior. Knowledge of the stability helps in selecting appropriate formulation and packaging as well as providing suitable storage conditions and shelf-life, which is essential for regulatory documentation [14].

The purpose of this research is to investigate the long-term stability and accelerated stability of two different types of pH-responsive DNGs, that is, FDNGs and GDNGs which were formulated using formaldehyde and glyoxal as a cross-linker, respectively. In addition, the effects of various types and quantities of cross-linker on stability were also studied. The changes of properties namely mean diameter, ζ-potential, chemical structure, drug remaining, pH-responsive behavior and amount of drug release in both DNGs after storage over a period of 6 months were evaluated in order to elucidate the optimal conditions for DNG stability during storage for future application.

2. Materials and methods

2.1. Materials

Dextrin (molecular weight of 1400 Da) was a gift from Siam Modified Starch Co., Ltd. (Pathumthani, Thailand). Glyoxal, ethanol and doxorubicin hydrochloride (DOX) were obtained from Sigma-Aldrich Chemie (Steinheim, Germany). Hydrochloric acid, formaldehyde and n-hexane were purchased from RCI Labscan (Bangkok, Thailand). Tween® 80 and Span® 80 were purchased from P.C. Drug Center Co., Ltd. (Bangkok, Thailand). Deionized water was used throughout the study.

2.2. Preparation of dextrin nanogels

DOX loaded-DNGs were prepared as described previously by our group [15] with some modifications. Water-in-hexane emulsions were prepared, in order to form a nanoemulsion template, using 7% (w/w) mixture of Span® 80/Tween® 80 as emulsifier. Dextrin and DOX were dissolved in the water phase to obtain the final concentration of 5% (w/w) and 0.2 mg/mL, respectively. The water phase was added to the emulsion template and ultrasonicated for 1 minute to form nanoemulsions. After the nanoemulsions were obtained, different concentrations of cross-linking agent (that is, formaldehyde or glyoxal) were added immediately to achieve mole ratios of dextrin to cross-linking agent of 4:1, 10:1, 15:1 and 20:1. The mixtures were homogenized via ultrasonication (UP400S, Hielscher, Germany) with 100% amplitude of ultrasound power (400 W, 24 KHz) for 30 min. The obtained nanoemulsions were then stirred with a magnetic stirrer for 12 h to continue the cross-linking reaction. DNGs were precipitated from the nanoemulsions by adding 99% (v/v) ethanol and washed 3 times with ethanol and finally rinsed with deionized water. Subsequently, the DNGs were freeze-dried for 24 h. The dried DNGs obtained from the freeze-drying process were packaged in zip-lock bags and kept at 4 °C until further analysis.

2.3. Stability study

The dried DNGs were kept under two conditions – 25 °C ± 2 °C/60% ± 5%RH (accelerated conditions; in stability chamber) and 5 ± 3 °C (long term condition; in refrigerator), for 6 months before further investigation.

2.4. Particle size and ζ-potential determination

Before measurement, DNGs were dispersed with phosphate buffer (pH 7.4, 6.8 and 5) and filtered through a 0.45-μm membrane. Zetasizer Nano-ZS (Malvern Instruments, UK) equipped with a He—Ne laser beam operating at a wavelength of 633 nm and a detector fixed at a scattering angle of 173°, was used to determine the hydrodynamic diameter and ζ-potential of DNGs at 25 °C. Measurements were performed three times.

2.5. Morphological observation of DNGs

Morphological analysis of DNGs was carried out on a transmission electron microscope (TEM; model JEM-1230, JOEL Corp., Japan). TEM analyses were performed by sample mounting on a copper glider grid of 3.5 mm with a single aperture, adsorbed with filter paper and dried at ambient temperature, prior to TEM examination.

2.6. ^{13}C nuclear magnetic resonance spectroscopy (^{13}C NMR)

The samples were dissolved in deuterium oxide. The ^{13}C NMR spectra of samples were recorded on NMR spectroscopy (model