International Journal of Biological Macromolecules 84 (2016) 31–34

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

journal homepage: www.elsevier.com/locate/ijbiomac

Short communication

Chitosan-based hydrogel nanoparticle amazing behaviors during transmission electron microscopy

Hajar Ashrafi [a,b], Amir Azadi [a,c,*]

[a] Pharmaceutical Sciences Research Center, School of Pharmacy, Shiraz University of Medical Sciences, Shiraz, Iran
[b] Department of Pharmaceutics, School of Pharmacy, International branch, Shiraz University of Medical Sciences, Shiraz, Iran
[c] Department of Pharmaceutics, School of Pharmacy, Shiraz University of Medical Sciences, Shiraz, Iran

ARTICLE INFO

Article history:
Received 10 July 2015
Received in revised form
16 November 2015
Accepted 30 November 2015
Available online 4 December 2015

Keywords:
Hydrogel nanoparticles
Transmission electron microscopy
Chitosan
Methotrexate

ABSTRACT

Nanogels are potential polymeric nanoparticulate systems of interest in biomedical applications, including time-controlled drug delivery and active drug targeting. With the aim of preparation of a nanocarrier for brain enhancement of the BBB-restricted hydrophilic drugs, nanogel loaded with the antineoplasm drug, methotrexate was prepared using an ionic gelation process. During transmission electron microscopy imaging, hydrogel nanoparticles were found as a polymeric matrix containing aqueous vacuoles. With emitting the electrons and increase in energy intake, the vacuoles were interconnected and form a large one. Then the volume of the new vacuole grew and subsequently decreased over the time. The behavior was in good agreement with drug release kinetic findings. These results provide important guidelines for designing top-down fabricated hydrogel nanoparticles with specific drug release kinetic mechanisms for enhancing and predicting the drug delivery efficacy.

1. Introduction

Hydrogel nanoparticles or in the other words nanogels as a group of nanoparticulate systems, have been a point of consideration as a promising transporter for drug-delivery purposes [1]. These nanogels have extensive potential and capability because they contain the characteristic features of the beneficial properties of hydrogels (because of their high degree of hydrophilicity) and nanoparticles (because of their small size) in drug delivery. In this way, it appears that world of science will benefit from features of hydrophilicity, flexibility, versatility, high water absorptivity, and biocompatibility of these particles and all the benefits of nanoparticles, fundamentally long life span in circulation and the possibility of being actively or passively targeted to the desired biophase, e.g., tumor sites [1,2]. Different methods have been reported to fabricate the nanogels. Other than the generally utilized synthetic polymers, active research has concentrated on the preparation of nanoparticles with natural hydrophilic polymers, such as chitosan (CS) [3].

CS, especially, CS nanoparticles offer many advantages because of their proper stability, low toxicity, simple and gentle preparation methods, and provide versatile routes of administration; they have gained more attention as a drug-delivery carrier [4,5]. CS has been widely studied in the preparation of nanoparticles for drug delivery and has ability to control the release of active agents [6–8]. Moreover, CS is a hydrophilic and positively charged polymer which enables to interact with negatively charged polymers, macromolecules, and even certain inorganic polyanion [4]. In this study, in situ TEM observations of the behavior of CS nanogels have been made. Experiments were conducted using a TEM sample holder permitting the evaluation of the nanogels under their natural aqueous suspended forms. The main purpose of this study is to see if the hydrogel nanoparticles can have the ability to change during electron bombardment.

2. Materials and method

2.1. Materials

ChitoClear® (a commercially accessible low molecular weight chitosan with level of deacetylation of 95%; viscosity of a 1% (w/v) solution, 6 mPa) was obtained from Primex (Siglufjorour, Iceland). Pentasodium triphosphate (Merck, Darmstadt, Germany) was purchased locally. Methotrexate was kindly supplied by Loghman

* Corresponding author at: Department of Pharmaceutics, School of Pharmacy, Shiraz University of Medical Sciences, Shiraz, P.O. BOX 71345-1583, Iran. Tel.: +98 71 32424256; fax: +98 71 32424256.
E-mail addresses: aazadi@sums.ac.ir, azadi_a@razi.tums.ac.ir (A. Azadi).

http://dx.doi.org/10.1016/j.ijbiomac.2015.11.089
0141-8130/

Pharmaceutical Co. (Tehran, Iran). All other chemicals, solvents and reagents utilized were of chemical or analytical grade, as required, and were obtained locally.

2.2. Preparation and characterization of hydrogel nanoparticles loaded by MTX

The hydrogel nanoparticles were prepared utilizing the ionotropic gelation technique, i.e., by means of ionic crosslinking of chitosan (CS) using a polyanion, TPP. In the early phase of the study, a systematic multi-objective optimization methodology was used to optimize the fabrication method of nanoparticles. For this reason, the hydrodynamic particle size, poly-dispersity index (PDI), loading efficiency and loading capacity of the resulting nanoparticles were evaluated simultaneously as the four responses expected to be optimized. In optimum condition, as the beginning stage, chitosan solutions with specified concentrations were prepared in sodium acetate buffer (final sodium acetate concentration of 5.54%, w/v; pH 4) under continuous mixing at 2000 rpm for 3 h. On the other hand, 1 ml of the polyanionic TPP solution (0.5%, w/v) was added to 7 ml of the polycationic chitosan solution (0.06%, w/v) in acetate buffer (pH 4) in a drop-wise way, over a 2 min time period under the constant magnetic stirring (1500 rpm) at 28 °C. Thereafter, the nanodispersion was stirred for an additional 60 min in order to cure the resulting nanoparticles. At last, nanogels were collected by means of centrifuging the specimens at 12,000 rpm for 15 min and re-dispersing them in distilled water. Incorporation of MTX [9–11] into the nanoparticles was performed by dissolving drug in the polyanion TPP solution to obtain sufficient final concentrations after mixing with an oppositely charged polymer solution during the preparation process.

Colloidal systems, especially nanoparticles, differ from their micro-sized analogues in terms of majority of physicochemical properties, which is started primarily from their sub-micron sizes. Therefore, a broad assessment of *in vitro* characteristics of the prepared nanostructures was made in this project which is reported with detailed information separately. Briefly, particle size distribution, polydispersity index (PDI), and zetapotential of the optimized nanoparticles (loaded and un-loaded) were measured by dynamic light scattering using a particle size/zeta analyzer (Malvern instrument, model Zetasizer 3000 HS, Malvern, Worcestershire, UK) at 25 °C with a detection angle of 90°. Nanogels were evaluated for *in vitro* drug release while suspended in phosphate-buffered saline (PBS; pH 7.4) utilizing a gadget planned and collected in-house. The device consisted of two Franz cells as the donor and receiver phase containers separated by a dialysis membrane (Dialysis Tubing Cellulose Membrane, Sigma–Aldrich, USA, Art No. D9527, cut-off 12 kDa). The vessels were double-jacketed with 37 °C water circulating between the jacket walls throughout the study. The concentration of MTX in the release medium was determined using the developed HPLC method.

2.3. Transmission electron microscopy

The morphology and possible aggregation of the nanogels was characterized using transmission electron microscopy (TEM) (Zeiss, model EM10C, Germany). For TEM imaging, samples were fixed on the copper grids, dried at room temperature and examined using TEM without being stained.

3. Results and discussion

Nanogels were fabricated via the ionotropic gelation procedure. This method is based on the ability of chitosan to generate a gel structure after contacting with polyanions such as TPP by forming inter- and intra-molecular linkages. In optimum condition, the particle size of MTX-loaded nanogels was 118.54 ± 15.93 nm. Furthermore, the size dispersity of the nanoparticle populations (PDI value) was 0.34. The zeta potential distribution diagrams of the hydrogel nanoparticles, while being mono-dispersed, showed an overall positive mean zeta potential, i.e. $+21.92 \pm 0.83$.

The size evaluation of samples by TEM, showed highly spherical shapes while confirming the size obtained by particle size analysis. In addition, the apparent hollow vacuole assembly of the nanogels as a typical behavior of the samples is noteworthy (Fig. 1). In fact, hydrogel nanoparticles were found as polymeric matrices containing aqueous vacuoles. Drug molecules are dispersed in polymeric matrix as well as water containing ponds. But it is surprising that during imaging, nanogels showed an interesting behavior. With emitting the electrons and increase in energy intake, the vacuoles were interconnected and form a large one. Then the volume of the new vacuole grew and subsequently decreased over the time. Finally the uniform polymeric particle was obtained. It seems that the electron bombardment led to an increase in free energy of water molecules in the small vacuoles. Based on the escaping tendency, this can be due to increased internal pressure increases the volume of the vacuole and join them together. After this, with the same explanation, the volume of the large vacuole will increase too. Then, due to the water vapor loss, a uniform polymer matrix remains in place. The *in vitro* drug (MTX) release profile and the release kinetics of drug from the nanogels were investigated in our recent study [12,13]. Generally, a kinetic study of drug release from a carrier is often attempted to recognize the main determinants of the drug release rate from the carrier with the ultimate goal of the identification of the ideal set of conditions leading to the desired release profile *in vivo*. To determine the best equation describing the drugs release profiles, all the experimental release data were fitted to eight mathematical models. The zero order model equation describes the systems where the drug release rate is not dependent on its remaining concentration. The first order release kinetics, in contrast, describes a system with drug release rate dependent upon the remaining drug concentration in each definite time. Higuchi model, describing a non-eroding homogenous granular matrix, proposes a direct relation of the drug release rate from the matrix to the square root of time and is basically derived from the Fickian diffusion. The Hixson-Crowell cube root model describes the cube root of the released amount of the drug linearly related to the time and is specifically applied to the systems which erode over time. Square root of mass and Three second root of mass models are two variants derived from the Hixson-Crowell cube root law to explain more accurately some specific release behaviors from the drug delivery systems. To describe drug release mechanism more precisely, there is a more comprehensive semi-empirical formula, called Korsmeyer-Peppas power law. The Weibull model is also an empirical model which has been widely applied to release data of both rapid and extended release drug delivery systems.

Sum square of errors (SSE), sum square of regression (SSR) and sum square of total variation (SST) were calculated to evaluate the coefficients of determination (R^2) for each model and the accuracy of the best fitted data by the suggested mathematical models.

The relative sizes of the sums of squares terms demonstrate how “good” the regression is in terms of fitting the calibration data. If the regression is “perfect”, then SSE is zero, and R^2 will be 1.

Also, the accuracy and predictability of the models were compared by calculation of absolute percent error (E) for each set as