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Synthesis, characterization, release kinetics and toxicity profile of drug-loaded starch nanoparticles

Mehrez E. El-Naggar[a,*], M.H. El-Rafie[a], M.A. El-sheikh[a], Gina S. El-Feky[b,c], A. Hebeish[a]

[a] Textile Research Division, National Research Centre (Affiliation ID: 60014618), 33 Behouth St., Dokki, Giza, Egypt
[b] Pharmaceutical Technology Department Centre (Affiliation ID: 60014618), National Research Centre, Egypt
[c] Pharmaceutics Department, Faculty of Pharmacy, October University for Modern Sciences and Arts, Egypt

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ABSTRACT

The current research work focuses on the medical application of the cost-effective cross-linked starch nanoparticles, for the transdermal delivery using Diclofenac sodium (DS) as a model drug. The prepared DS-cross-linked starch nanoparticles were synthesized using nanoprecipitation technique at different concentrations of sodium tripolyphosphate (STPP) in the presence of Tween 80 as a surfactant. The resultant cross-linked starch nanoparticles loaded with DS were characterized using world-class facilities such as TEM, DLS, FT-IR, XRD, and DSc. The efficiency of DS loading was also evaluated via entrapment efficiency as well as in vitro release and histopathological study on rat skin. The optimum nanoparticles formulation selected by the JMP® software was the formula that composed of 5% maize starch, 57.7 mg DS and 0.5% STPP and 0.4% Tween 80, with particle diameter of about 21.04 nm, polydispersity index of 0.2 and zeta potential of −35.3 mV. It is also worth noting that this selected formula shows an average entrapment efficiency of 95.01 and sustained DS release up to 6 h. The histophathological studies using the best formula on rat skin advocate the use of designed transdermal DS loaded cross-linked starch nanoparticles as it is safe and non-irritant to rat skin.

The overall results indicate that, the starch nanoparticles could be considered as a good carrier for DS drug regarding the enhancement in its controlled release and successful permeation, thus, offering a promising nanoparticulate system for the transdermal delivery non-steroidal anti-inflammatory drug (NSAID).

1. Introduction

Developing suitable drug delivery systems targeted toward transdermal disease is one of the major focuses of pharmaceutical scientists. There are several new transdermal drug delivery systems under investigation such as: hydrogels [1]; microparticles [2]; nanoparticles [3–6]; liposomes [7]; collagen shields [8–10]; ocular inserts/discs [11]; and dendrimers [12]. The most promising of all the developed over the past 25 years of intense research in medical therapeutics are the nanoparticles due to their sustained release and prolonged therapeutic benefit [13]. Nanoparticles for drug delivery are part of a relatively new research field called nanobiotechnology [14]. The role of nanoparticles is to minimize adverse systemic effects of medical treatments by protecting, targeting, and finally releasing a drug at a specific site in the body. For that purpose, nanoparticles are for example designed to interact with biological interface through specific target–ligand interactions. Nanoparticles are solid colloidal particles made of natural or artificial polymers ranging in size from 10 to 1000 nm [15], in which drug can be dissolved, entrapped, adsorbed or covalently attached [16]. Colloid size is an important property that affects the ability of systems to deliver drugs effectively. Particles having sizes greater than 1 μm are too large to diffuse passively through epithelial membranes; thus, typically, they remain at the site of administration. If the size of the particles exceeds ca. 250–300 nm, then most will be captured by filtration in the red pulp of the spleen and phagocytosed within the cells of the reticuloendothelial system. The fate of particles is determined by their size and surface character [17].

Transdermal delivery for many drugs could avoid many problems associated with the oral route such as the drastic change of pH, the presence of enzymes, variable transit times and fluctuating drug plasma concentrations. In addition, transdermal delivery offers longer duration of action resulting in a reduction in dosing

* Corresponding author. 33 Behouth st. Dokki. Giza, Egypt.
Tel.: +201126642204, 0020233357807.
E-mail addresses: mehrez_chem@yahoo.com, melnagg@ncsu.edu (M.E. El-Naggar).

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frequency, improved bioavailability, reduced side effects as well as flexibility of terminating the drug administration by simply removing the patch from the skin. Regarding to transdermal therapy, the key point of dosage design is to enhance the controlled release of soluble drug and improve its permeability via skin without causing irritation.

Diclofenac sodium (DS) is a non-steroidal anti-inflammatory drug (NSAIDs). Globally, it is the most widely used class of therapeutic drugs and is a suitable candidate for the development of sustained and/or controlled release products [18]. The NSAID DS (DS: 2-[(2,6-dichlorophenyl)amino]benzeneacetic acid) is widely used in the treatment of rheumatoid disorders and other chronic inflammatory diseases [19,20].

Starch is an abundant, non-toxic, biodegradable, edible, and relatively inexpensive material. Starch has the ability to be cross-linked on contact with polyanions due to the formation of inter- and intramolecular cross-linking mediated by these polyanions. Among some polyanions investigated, sodium tripolyphosphate (STPP) is the most popular as effective cross-linking agent [21,22]. STPP is a non-toxic solid with no reported adverse effects on humans [23]. The starch–STPP nanosystems exhibit some attractive features. One of these is the ease of preparation under mild condition which render them promising carriers for drug delivery [24].

The simplest method to prepare DS loaded starch nanoparticles is the solvent displacement method also known as nanoprecipitation method, developed by Fessi et al. [25]. The method is based on the interfacial deposition of a polymer following displacement of a semi-polar solvent miscible with water. Low complexity and low energy consumption of this technique as well as being widely applicable without any additives make it suitable for the manufacturing of defined nanospheres [26]. However, cross-linked starch nanoparticles loaded with DS fabricated by nanoprecipitation technique for the controlled release in medical applications presents some challenges, particularly when toxic organic solvents are used as dissolving system. These organic solvents might affect the stability of the encapsulated bioactive compounds and leave toxic residues incompatible with medical applications.

Products safety and stability concerns restrict the use of these toxic organic solvents thereby making it necessary to develop an alternative “safe” process. An attractive method based on the phenomenon that in aqueous polymer systems phase can occur. In this method, an aqueous solution of a water-soluble polymer is emulsified as a dispersed phase in an aqueous solution of sodium hydroxide as a continuous phase. Subsequently, the dispersed polymer phase is cross-linked to form nanospheres.

In the present work, cross-linked starch nanoparticles were selected as the carrier of choice, and these nanoparticles loaded with DS were prepared by nano-precipitation method without using toxic organic solvents. The work will be carried out according to the following steps: Studying the effect of STPP as cross-linking agent and DS concentrations on the prepared starch. A two-level factorial design experiment will be used for the prediction of optimized formulation for cross-linked starch nanoparticles loaded with DS. The formulated nanospheres will be systematically characterized by drug content, encapsulation efficiency, size distribution, Transmission electron microscopy (TEM), polydispersity index (PDI), and zeta potential (ZP) for shape and surface characters, and in vitro release studies. The formulated nanospheres will be analyzed by making use of physicochemical characterization using Fourier transform infrared spectroscopy (FT-IR), X-ray diffraction (XRD) and Differential scanning calorimetry (DSC) to find out the physical nature (crystallinity), thermal behavior, possible occurrence of interaction between DS and cross-linked starch nanoparticles. In vivo targeting efficiency of the optimum formula of starch cross-linked with STPP and loaded with DS will be tested histopathologically in skin irritation of healthy rats.

2. Materials and method

2.1. Materials

Starch was kindly supplied by Starch and Glucose Company, Egypt. Diclofenac sodium (DS) was kindly gifted by Delta Pharma, 10th of Ramadan, Egypt. Tween® 80 (polyoxythylene sorbitan monoleate) > 99.9% was obtained from Sigma–Aldrich Chemie GmbH (Germany). Sodium tripolyphosphate (STPP) was purchased from Sigma–Aldrich Chemical Co. Ltd. Phosphate buffer solution (PBS) was prepared using disodium hydrogen orthophosphate, and potassium dihydrogen orthophosphate. Carbopol powder was supplied by Across Company (New Jersey, USA). Sodium hydroxide and absolute ethyl alcohol were of analytical grade and were used as received. All solutions are prepared using deionized water.

2.2. Methods

2.2.1. Preparation of cross-linked starch nanoparticles loaded with DS

Cross-linked starch nanoparticles loaded with DS were fabricated by nano-precipitation technique modified as follows: 5% native maize starch was dispersed in 70 ml distilled water containing 30% sodium hydroxide (based on weight of native maize starch), which performed as dispersed phase under continuous high speed homogenization for 30 min at 25 °C. 0.4 g of Tween® 80 dissolved in 20 ml distilled water containing different amount of DS (20, 50 and 100 mg) was added drop wise to the dispersed starch under high homogenization speed. After 30 min, 10 ml distilled water containing different amounts of STPP (0.5, 1, and 2 g) as cross-linking agent was added gently to the previous solution, keeping in mind that, the total volume of the reaction mixture is 100 ml. The reaction mixture was kept stand at room temperature for 2 h with agitation to effect cross-linking with constant agitation rate at 25 °C. The resulting DS encapsulated cross-linked starch nanoparticles were subsequently precipitated by 100 ml of absolute ethanol. The resultant powder was purified by means of centrifugation and washing, rinsed twice with 80/20 absolute ethanol/water to remove unreacted compounds and finally with absolute ethanol. Then the resultant nanoparticles were isolated by means of centrifugation for 1 h at 4500 rpm. At the end, the supernatant was then taken for further analysis to determine the loss in DS. The supernatant was freeze-dried for 12 h and kept in closed containers for further analysis. The as described freeze-dried DS loaded cross-linked starch nanoparticles in the solid state can be easily re-dispersed in distilled water by hand agitation before use (Scheme 1).

2.2.2. Factorial design

Different DS loaded starch nanoparticle formulations were prepared based on the 3^2 factorial design [27]. Percentage of the cross-linking agent ($X1$) and that of the drug ($X2$) in the formulation were selected as two independent variables. Three levels of each variable were selected and possible batches were prepared using different levels of variables. JMP® (version 7, SAS, USA) was used to obtain values of the coefficients in the equation and f statistics were used to identify statistically significant terms.

2.3. Characterization

2.3.1. Entrapment efficiency (EE%) of nanoparticles

The nanoparticles suspension was centrifuged at 4500 rpm for 60 min. The supernatant solution was separated. 1 ml of