European Journal of Medicinal Chemistry 60 (2013) 249–253

Contents lists available at SciVerse ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Predictive modeling of insulin release profile from cross-linked chitosan microspheres

S. Jose [a,**], J.F. Fangueiro [b], J. Smitha [a], T.A. Cinu [a], A.J. Chacko [a], K. Premaletha [c], E.B. Souto [b,d,*]

[a] Department of Pharmaceutical Sciences, Mahatma Gandhi University, Cheruvandoor Campus, Ettumanoor 686 631, Kerala, India
[b] Faculty of Health Sciences, Fernando Pessoa University, Rua Carlos da Maia, 296, P-4200-150 Porto, Portugal
[c] Academy of Pharmaceutical Sciences, Pariyaram Medical College, Kannur, Kerala, India
[d] Institute of Biotechnology and Bioengineering, Centre of Genomics and Biotechnology, University of Trás-os-Montes and Alto Douro (CGB-UTAD/IBB), P.O. Box 1013, P-5001-801 Vila Real, Portugal

ARTICLE INFO

Article history:
Received 9 March 2012
Received in revised form
10 November 2012
Accepted 7 December 2012
Available online 19 December 2012

Keywords:
Insulin
Bacitracin
Chitosan
Medicinal chemistry
Higuchi model
Korsmeyer–Peppas model microspheres
Non-Fickian diffusion
Oral delivery

ABSTRACT

Insulin-loaded microspheres composed of chitosan 3% (w/v), and loading 120 IU insulin were produced by emulsion cross-linking method. Cross-linking time was 5 h and glutaraldehyde 3.5% (v/v) was used as cross-linker. Swelling ratio studies were evaluated to predict release of insulin from chitosan microspheres. Bacitracin and sodium taurocholate were incorporated in the formulations as proteolytic enzyme inhibitor and absorption enhancer, respectively. In vitro insulin release studies were performed in phosphate buffer pH 7.4 and also in HCl pH 2 with and without trypsin. Activity of bacitracin was also evaluated. In vitro release showed a controlled profile up to 12 h and the formulation containing 0.15% (w/v) of bacitracin revealed a maximum biological activity of about 49.1 ± 4.1%. Mathematical modeling using Higuchi and Korsmeyer–Peppas suggested a non-Fickian diffusion as the mechanism of insulin release. Insulin-loaded chitosan microspheres for oral delivery showed to be an innovative and reliable delivery system to overcome conventional insulin therapy.

1. Introduction

Chitosan microspheres are extensively used to provide a controlled release of peptides and proteins, such as insulin [1]. This delivery system enhances the peptide bioavailability, as well as the uptake of other hydrophilic substances through epithelial layers with improved protection against the harsh environment of stomach upon oral administration [1,2]. Chitosan is a polymer with mucoadhesive properties that increases the contact with mucosa, where drugs are absorbed, resulting in a concentration gradient in favor of its absorption [3–5]. In vitro profiles of drugs are important for the development of new drug delivery systems as an assessment of bioequivalence. The drug release profile is linked to the system's properties and determines the amount of drug available for absorption [6]. Aspects related to drug absorption, biodistribution, metabolism and elimination (the so-called ADME processes) are therefore of upmost relevance to predict the efficiency of delivery systems by in vitro studies. The type of drug and matrix, its polymorphic form, crystallinity, particle size, and solubility can influence the release profile [7]. Models of drug release are often applied to predict controlled release in delivery systems such as chitosan microspheres; however, it does not provide any information on the mechanisms that control the process. The use of empirical models for simulating drug release profiles is a function of time related to the amount of drug released from the pharmaceutical dosage form [8]. The quantitative values are interpreted by generic equations that mathematically translate the release curve. To understand dissolution profiles, mathematical models and statistical analysis are used [9]. To describe the insulin releasing mechanism from chitosan microspheres, release profiles were analyzed applying 4 different mathematical models, i.e. zero order, first order, Higuchi and Korsmeyer–Peppas. In the zero order kinetics, drug dissolution profiles is slow as pharmaceutical

* Corresponding author. Faculty of Health Sciences of Fernando Pessoa University, Rua Carlos da Maia, 296, Office S.1, P-4200-150 Porto, Portugal. Tel.: +351 22 507 4630x3056; fax: +351 22 550 4637.
** Corresponding author.
E-mail addresses: eliana@ufp.edu.pt, sajanjose@hotmail.com (E.B. Souto).

0223-5234/$ – see front matter © 2012 Elsevier Masson SAS. All rights reserved.
http://dx.doi.org/10.1016/j.ejmech.2012.12.011

systems do not disaggregate, assuming no changes in dissolution area which is maintained under equilibrium. The equation translating this kinetic model is:

$$Q_1 = Q_0 + K_0 t$$

where Q_1 is the amount of drug dissolved in time t, Q_0 is the initial amount of drug in the solution (generally, $Q_0 = 0$) and K_0 is the zero order release constant. The graphic of drug dissolved versus time will be linear.

In first order kinetics, the dissolution phenomenon of a solid particle in a liquid media implies a surface action and from several deductions. The equation in decimal logarithms can be depicted as:

$$\log Q_t = \log Q_0 + \frac{K_1 t}{2.303}$$

where Q_t is the amount of drug released at time t, Q_0 is the amount of drug in the solution, and K_1 is the first order release constant. The graphic of the decimal logarithm of the released amount of drug versus time will be linear. This model is usually applied for dissolution profiles of water-soluble drugs in porous matrices.

Higuchi model is based on the quantification of drug release derived from a very simple equation. This model describes a direct proportionality between the cumulative amount of drug released overtime based on a pseudo-steady-state approach [10–12]. For Higuchi model, the drug release can be defined as the mass transfer of drug molecules from the dosage form to the surrounding medium. Higuchi equation allows device optimization, underlying drug release mechanisms [12]. Higuchi model describes the release of drugs as the square root of time based on the Fickian diffusion and the simplified equation is:

$$f_t = K_H \cdot t^{\frac{1}{2}}$$

where K_H is the constant reflecting the design variables of the system [13].

Korsmeyer–Peppas model is based on the Fick's Law. This model is used to describe the release of the solute when the prevailing mechanism is a combination of drug diffusion – Fickian transport-, and in Case II transport – non-Fickian-, controlled by the relaxation of polymer chains [13,14]. Korsmeyer–Peppas model is useful to predict the release mechanism in the first 10 h being described by the following equation:

$$\frac{M_t}{M_\infty} = K \cdot t^n$$

where M_t/M_∞ is the fraction of drug released at time t, K is the rate constant, and n is the diffusion exponent. According to this model, the value of n identifies the release mechanism of drug. Values of n between 0.5 and 1.0 indicate anomalous transport kinetics, n approximately 0.5 indicates the pure diffusion controlled mechanism (Fickian diffusion). The smaller n values below 0.5 may be due to drug diffusion partially through a swollen matrix and water filled pores in the formulations [15–17]. This model is often applied for the analysis of the release profile of drugs in polymeric systems.

The purpose of this study was to evaluate the insulin release profile of insulin-loaded chitosan microspheres, and understand which model this system follows. Selected models were previously confirmed to fulfill all conditions for their use in chitosan microspheres. Swelling ratio studies were also evaluated as a function of pH. The effect of a protease inhibitor, such as bacitracin, in the release profile of insulin from chitosan microspheres was evaluated. Bacitracin is a well-known antibiotic used as inhibitor of all classes of proteases, being also resistant to enzyme digestion [18]. The use of a protease inhibitor could lead to a higher absorption of insulin in the gut [19,20], since it avoids peptide degradation and consequently its inactivation. For this purpose, the evaluation of the release of insulin in the presence of bacitracin is an important parameter to predict the absorption later in the intestine. In addition, an absorption enhancer, i.e. sodium taurocholate, was used to optimize the release profile of insulin from chitosan microspheres. Sodium taurocholate is a bile salt simple micellar system that enhances absorption in the gastrointestinal tract and avoids degradation of insulin by mucus and homogenates providing permeation-enhancing and enzyme-inhibiting effect [21,22]. The aim of this work was to prove the ability of insulin-loaded chitosan microspheres to release the peptide following an improved profile in terms of amount of release insulin and the evaluation of their biological activity in terms of the ratio of insulin released by two procedures.

2. Materials and methods

2.1. Materials

Chitosan was a gift sample from CIFT (Cochin, India). Human insulin injection (recombinant DNA origin 40 IU/ml) was obtained from Wockhardt Ltd. (Aurangabad, India). Bacitracin was purchased from Himedia laboratories Pvt. Ltd. (Mumbai, India). Sodium taurocholate was purchased from Loba Chemie Pvt. Ltd. (Mumbai, India). Glacial acetic acid and span 80 were purchased from Central Drug House (New Delhi, India). Light liquid paraffin, glutaraldehyde, hydrochloric acid (HCl), sodium hydroxide (NaOH), potassium dihydrogen phosphate (KH_2PO_4), petroleum ether and acetone were purchased from Nice Chemicals Pvt. Ltd. (Cochin, India). All other reagents were of analytical grade.

2.2. Methods

2.2.1. Preparation of chitosan microspheres

Chitosan microspheres were produced by emulsion cross-linking method. Briefly, chitosan solution 3% w/w was prepared using 4% (w/v) aqueous glacial acetic acid by overnight stirring in a magnetic stirrer. Insulin was dispersed in this solution and mixed. A volume of 3 ml of the mixture was added into 20 ml of oil phase containing Span®80 1% (v/v) and light liquid paraffin and then stirred by digital mechanical stirrer (Kemi, India) at 2–8 °C using an ice bath and at 1000 rpm to form a w/o emulsion. After 30 min of homogenization, 3.5 ml of glutaraldehyde (25% v/v) was added, stepwise. It was then left for stabilization and cross-linking for a period of 5 h. The obtained microspheres were then centrifuged using a centrifuge (Remi Equipments Ltd., India) at 4000 rpm and the sediment was washed with petroleum ether and acetone thrice, dried at room temperature and stored in refrigerator at 2–8 °C.

2.2.2. Particle size analysis

The size of microspheres was analyzed by optical microscope (Olympus India Pvt. Ltd., India) fitted with a calibrated eyepiece micrometer. The particle diameters of about 100 microspheres were measured randomly. Samples were placed on the slide and analyzed without further treatment.

2.2.3. Swelling studies

Swelling behavior of insulin-loaded chitosan microspheres were evaluated as a function of pH. Insulin-loaded chitosan microspheres were put in a solution of pH 2 (HCl 0.1 N) at 37 °C. The particle size increase observed during swelling continued even after 12 h. The swelling ratio (G) was measured applying the following equation: