International Journal of Biological Macromolecules 50 (2012) 905–909

Contents lists available at SciVerse ScienceDirect

International Journal of Biological Macromolecules

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

Effect of membrane dialysis on characteristics of lyophilised chitosan wafers for potential buccal delivery of proteins

Isaac Ayensu [a,b], John C. Mitchell [a], Joshua S. Boateng [a,*]

[a] Department of Pharmaceutical, Chemical and Environmental Sciences, School of Science, University of Greenwich at Medway, Chatham Maritime, Kent ME4 4TB, UK
[b] Department of Pharmaceutical Chemistry, Faculty of Pharmacy and Pharmaceutical Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana

ARTICLE INFO

Article history:
Received 2 January 2012
Received in revised form 4 February 2012
Accepted 13 February 2012
Available online 21 February 2012

Keywords:
Buccal delivery
Chitosan
Lyophilised wafers
Membrane dialysis
Mucoadhesion
Protein delivery

ABSTRACT

The effect of membrane dialysis on the characteristics of chitosan based lyophilised wafers was investigated. Gels loaded with BSA, glycerol and D-mannitol were lyophilised with or without membrane dialysis and characterised by X-ray diffraction, attenuated total reflectance Fourier transform infra red spectroscopy, circular dichroism, scanning electron microscopy, hydration capacity, *in vitro* mucoadhesivity and drug dissolution. The dialysed wafers demonstrated enhanced mucoadhesion and drug release properties while newly formed sodium acetate in the undialysed wafers caused increased crystallinity with poor mucoadhesion and drug release properties. Removal of sodium acetate by membrane dialysis is essential for obtaining optimised wafers for potential application to the buccal mucosa surface.

© 2012 Elsevier B.V. All rights reserved.

1. Introduction

Chitosan is a weak cationic polyaminosaccharide and natural biopolymer derived from deacetylation of chitin [1]. It has recently received increased attention due to its biocompatible, biodegradable, non-toxicity, hydrophilicity and excellent lyophilised wafer and film-forming properties [2]. Its application as a functional material extends across several industries including agro-processing, food, pharmaceutical and medicinal industries [3,4] as well as environmental protection, biotechnology and cosmetics industries [5,6]. The use of chitosan in appropriate matrix formulations for the controlled release of drugs spans over a decade [7]. A comparison between chitosan and other commonly used polymeric excipients indicates that the cationic polymer has higher bioadhesivity compared to other natural polymers, such as cellulose, xantham gum and starch [8]. In addition, the permeation enhancing and peptidase inhibition properties of chitosan and its thiolated derivatives have been recognised as outstanding characteristics required for the delivery of proteins and peptide drugs via the buccal mucosa [9]. Microcrystalline chitosan as a gel-forming excipient for matrix-type drug granules has been studied and it was observed that crystallinity, molecular weight and degree of deacetylation affected the release rates from the chitosan-based granules [10]. The antimicrobial activities exhibited by chitosan [11] and chitosan oligomers [12] have been linked with their solubility in acidic media such as acetic acid [13] and hydrochloric acid [14].

The effective utilisation of chitosan has, however, been hampered by its poor solubility in neutral or alkaline media due to its dense crystalline structure [15]. Chitosan is, however, soluble in dilute aqueous solvents of up to a pH of 6.5 where the soluble $R\text{-}NH_3^+$ is produced by protonation of the glucosamine unit [16]. Methods investigated to overcome the insolubility of chitosan include protonation of the primary amine group and derivatisation by chemical modification [17]. Wang et al. [18] have also reported a method that has been investigated with ultraviolet absorption which improves the solubility of chitosan by employing a mild and convenient acylation reaction in neutral aqueous media.

Lyophilised wafers, prepared by freeze-drying polymer gels to obtain porous cakes can potentially be applied to mucosal surfaces for both local and systemic delivery of pharmacological agents such as proteins. The lyophilisation of protein formulations to obtain stable products has several benefits including extension of shelf life, storage at room temperature and improved patient compliance [19]. The porous nature of wafers provides the advantage of high drug loading capacity compared to films [20] and other semi-solid polymer gels [21].

In this paper, we report the effect of membrane dialysis as a process step on the physico-mechanical properties of protein loaded lyophilised chitosan wafers, as potential drug delivery systems

* Corresponding author. Tel.: +44 208 331 8980; fax: +44 208 331 9805.
E-mail addresses: j.s.boateng@gre.ac.uk, joshboat@hotmail.com (J.S. Boateng).

0141-8130/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijbiomac.2012.02.014

to the buccal mucosa membrane. Wafers were characterised for chemical structure, crystallinity, microscopic structure and *in vitro* mucoadhesive properties using X-ray diffraction (XRD), attenuated total reflectance-Fourier transform infra red spectroscopy (AT-IR), circular dichroism (CD), scanning electron microscopy (SEM) and texture analysis (TA), respectively. The resulting data were used to evaluate formulations prepared with or without dialysis. The effect of membrane dialysis on the functional characteristics of the wafers including mucoadhesion and drug release was studied.

2. Experimental

2.1. Materials

Chitosan (medium molecular weight, 190–310 kDa, 75–85% deacetylated), BSA (mol wt ~66 kDa), glycerol, D-mannitol, coomassie blue (Bradford reagent) and mucin (from bovine submaxillary gland, Type I-S) were obtained from Sigma (Gillingham, UK). Sodium acetate (anhydrous) and pulverised gelatine were purchased from Fischer Scientific (Loughborough, UK). All other reagents were of analytical grade and were used without further purification.

2.2. Formulation and lyophilisation

500 mg of chitosan was dissolved in 50 mL of 1% (v/v) acetic acid and the pH was adjusted to 6 with 5 M NaOH. The resulting gel was dialysed against 5 mM HCl five times, with Spectra/Por® Float-A-Lyzer® G2 dialysis device (8–10 kDa molecular weight cut-off; Sigma Gillingham, UK) [22]. The dialysed gel was loaded with 50% BSA and 10% each of plasticiser (glycerol) and cryoprotectant (D-mannitol) (per polymer weight) and stirred continuously for 15 min to obtain a homogeneous gel and kept at room temperature to remove all air bubbles. The process was repeated excluding the dialysis step. 3.0 g each of the uniform mixture was transferred into 12-well moulds (diameter 23 mm) (Thermo-Fisher Scientific Nunc, Leicester, UK) and lyophilised, applying an annealing temperature of −20 °C (3 h) during the freezing phase (−45 °C). Primary and secondary drying were at −25 °C and 20 °C, respectively, with a vacuum of 20 mtorr for 34 h on a Virtis adVantage XL 70 freeze dryer (Biopharma Process Systems, Winchester, UK). Dialysed drug loaded wafers were designated wafer (A) and the corresponding undialysed wafers designated wafer (B). All wafers were kept over anhydrous silica gel in desiccators till ready for characterisation.

2.3. Analytical characterisation of wafers

XRD studies of compressed lyophilised wafers (0.5 mm) were determined with D8 ADVANTAGE X-ray diffractometer (Bruker AXS GmbH, Karlsure, Germany). Transmission diffractograms were acquired with DIFFRAC plus XRD Commander over a start to end diffraction angle of $2\theta = 5–45°$, step size of 0.02° and a scan speed of 0.4 s. The operating conditions were 40 kV and 40 mA with CuKα radiation. Data were processed with EVA software for native BSA powder, chitosan powder, wafer (A) and wafer (B).

AT-IR spectra of sodium acetate (NaAc), BSA powder, chitosan powder, wafers (A) and (B) were acquired on an Excalibur series FTS 3500 ARX Fourier transform infrared spectrophotometer, equipped with Specac Golden gate (Varian, Oxford, UK). Win IR PRO software of wave number range 4000–600 cm^{-1} was used for the analysis. The spectra were collected at a resolution of 1.5 cm^{-1} with 16 scans per spectrum. A background spectrum was acquired and assigned for use on subsequent spectral acquisitions for each sample.

The conformational structure of BSA was examined by far-UV CD. Spectra of a 1 mg/mL solution of native BSA in 0.1 M PBS (pH 6.8) as the negative control or BSA released from the wafers were recorded at 25 °C from wavelength range $240 \geq \lambda \geq 190$ with bandwidth 1 nm, time-per-point 0.5 s and cell path-length of 0.1 mm using CD spectroscopy (Chirascan, Applied Photophysis). The mean residue ellipticity $[\theta]_{mrw,k}$ was determined using Eq. (1) below:

$$[\theta]_{mrw,k} = MRW \times \theta_k / 150 \times d \times c \quad (1)$$

where MRW is the mean residue weight, θ_k is the observed ellipticity (degrees) at wavelength k, d is the pathlength (cm) and c is the concentration (g/mL) [23].

Morphological studies were conducted with SEM. Wafers were placed on double sided adhesive carbon tape on labelled stainless steel stubs with external surfaces on the exposed side of the adhesive, sputter coated for 2 min at 30 mA and 1 kV with gold (Edwards Sputter Coater S150B) and placed in the chamber of a Cambridge Stereoscan S-360 SEM (Class one equipment, London, UK). Images (magnification 200×) acquired at an accelerating voltage of 20 kV and working distance of 15 mm were processed with i-scan2000 software.

2.4. Hydration capacity

Samples ($n = 4$) were placed in 25 mL of 0.1 M PBS solution (pH 6.8 ± 0.1 simulating salivary pH) at 37 ± 0.1 °C. The wafers were weighed initially and the hydration behaviour observed at fixed time intervals [1]. The surface-adhered liquid droplets were blotted off carefully between tissue papers and then reweighed to a constant weight. The hydration capacity was calculated as below:

$$\text{Hydration capacity (\%)} = 100 \times \frac{(Ds - D)}{D} \quad (2)$$

where Ds is the weight of the swollen wafer and D is the initial weight of wafer.

2.5. In vitro mucoadhesion studies

In vitro mucoadhesion studies using solid gel gelatine equilibrated with 2% mucin solution as mucosal substrate was performed on the wafers with a TA.HD.*plus* Texture Analyser (Stable Micro Systems, Surrey, UK), fitted with a 5 kg load cell in tension mode [24]. The wafer ($n = 4$) attached to a 75 mm diameter probe was set to approach the model mucosal substrate using the following settings: pre-test speed 0.5 mm/s; test speed 0.5 mm/s; post-test speed 1.0 mm/s; applied force 1 N; contact time 60.0 s; trigger force 0.05 N and return distance of 10.0 mm. Texture Exponent 32 software was employed to process the data. The peak adhesive force (PAF) required to detach wafer from the mucosal surface was determined, the area under the curve (AUC) representing total work of adhesion (TWA) was estimated from force–distance plot whiles the cohesiveness was assessed by the travel distance.

2.6. In vitro BSA release profiles

The wafers were immersed in beakers containing 25 mL of 0.1 M PBS (pH 6.8 ± 0.1) solution as release medium at 37 ± 0.1 °C and stirred at 150 rpm with a magnetic stirrer. The wafer remained on the surface of the dissolution medium throughout and therefore avoided contact with the magnetic follower at the bottom of the beaker. 50 μL of the release medium was withdrawn at fixed time intervals and replaced with fresh medium to maintain a constant volume. 1 mL of Bradford's reagent was added to the sampled release medium and the absorbance measured at 595 nm and 450 nm [25]. Both wafers were also assayed for BSA content by dissolving in 1% (w/v) acetic acid and analysed by Bradford assay. The amount of BSA released from the wafers was estimated from the linearised calibration curve ($r^2 > 0.99$) and the cumulative percentage drug release profiles of BSA were plotted. The cumulative % drug

Download English Version:

https://daneshyari.com/en/article/1987182

Download Persian Version:

https://daneshyari.com/article/1987182

Daneshyari.com