Colloids and Surfaces B: Biointerfaces 154 (2017) 48–54

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

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

Controlled release of insulin from folic acid-insulin complex nanoparticles

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ARTICLE INFO

Article history:
Received 21 July 2016
Received in revised form 28 February 2017
Accepted 1 March 2017
Available online 3 March 2017

Keywords:
Folic acid
Insulin delivery
Nanoparticle
HPLC
Controlled release

ABSTRACT

Associative interactions between folic acid and proteins are well known. This work leverages these interactions to engineer folic acid nanoparticles for controlled release of insulin during diabetes therapy. The insulin-loaded folic acid nanoformulation is synthesized during this study to achieve better insulin loading and encapsulation than previous strategies. The maximum insulin loading in the FA particles was kept at 6 mg with less than 10% insulin loss during the synthesis process which is significantly better compare to previous strategies. The folic acid nanoparticles of 50–150 nm size are further characterized in the present study. The release behaviour of insulin from the nanoparticles has been studied to quantify released insulin and folic acid with time using high performance liquid chromatography. Insulin release results suggest that more than 90% of the insulin is encapsulated and released within 24 h from folic acid nanoparticles. The analysis of folic acid release along with insulin release indicates that the particles are formed by folic acid-insulin complexation at the molecular level. The release of insulin from nanoparticles is controllable with the change in the crosslinking salt concentration as well as the amount of folic acid loaded during particle synthesis. These results prove that folic acid nanocarriers are capable to control the release of therapeutic proteins.

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1. Introduction

The advances in biotechnology have contributed significantly in the field of protein therapeutics [1]. Insulin is one of such protein which has been engineered for diabetes therapy in different activities and release models [2]. The early degradation of insulin in the stomach environment restricts the oral administration of insulin in the human body [3]. Currently, insulin is administered to diabetes patients through injections. Pain and inconvenience caused by multiple parenteral administration encourage the development of an oral formulation for insulin delivery [4]. Oral delivery of protein through nano-carrier may also help to target the sites in the body [5–9]. Another advantage of drug delivery through nanoparticles is controllable drug release [10]. Sustained drug delivery strategy can provide the required drug level in the body for longer time periods with fewer side effects [4]. Therefore, controlled drug release is desirable during the treatment of diabetes and several other autoimmune diseases where therapeutic proteins are used as medicine [11,12]. Various nano carriers are engineered for the effective controlled release of protein drug but problems such as burst release, high drug loss and low drug loading are common in those nanoformulation [13–16].

Proteins display associative interactions with a vitamin called folic acid (FA) [17–19]. FA is a biologically active molecule which is used in various drug delivery systems for targeting cells, fluorescence emission and increasing the bioavailability of drug [20–24]. Past investigations have shown the importance of self-assembled FA as an efficient carrier to provide controlled release of a model protein, cancer and tuberculosis drugs [25–30]. Ordered aggregates of FA loaded up to 57% drug have shown significantly less (about 10%) drug losses during particle synthesis [25–27,31,32]. This compares very well with other protein delivery strategies such as polymeric nanoparticles or liposome nanoparticles that have shown lower levels of loading from 0.1% to 33% with significant drug losses during processing [33–39]. Hence, we are interested in developing a protein delivery system with the help of associative interaction between FA and insulin to achieve high loading, low processing losses, and control over the insulin release.

In this study, insulin encapsulated FA nanoparticles are designed in a similar manner as in the previous study of FA nanoparticles with model protein [32]. During nanoparticle synthesis, the loading of FA and insulin has been maintained such that the ratio of moles of

Abbreviations: DI, deionised; DLS, dynamic light scattering; FA, folic acid; HPLC, high performance liquid chromatography; h, hours; HPMC, hydroxypropyl methylcellulose; PBS, phosphate buffer saline; TEM, transmission electron microscope; TFA, trifluoroacetic acid.

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http://dx.doi.org/10.1016/j.colsurfb.2017.03.002
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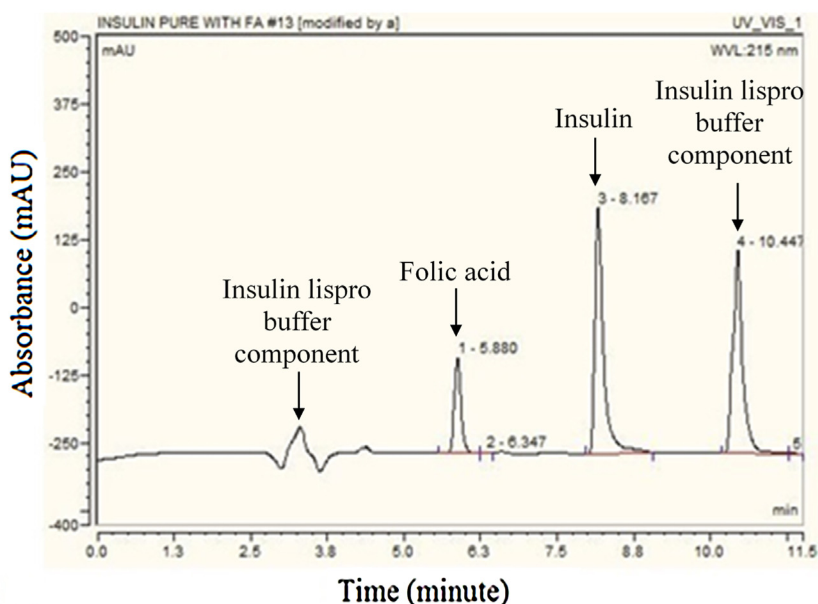

Fig. 1. Chromatogram of FA-insulin lispro mixture from HPLC.

aromatic ring residues in the structure of the protein to moles of FA loaded should be within the same range as in the previous study with model protein [32]. An important role of aromatic rings in FA-protein association is the reason to choose FA to protein ratio on the basis of the aromatic ring in the structure of protein and FA during particle synthesis. The particle size of the synthesized nanoparticles was analysed using dynamic light scattering (DLS) and transmission electron microscope (TEM). A method was established for quantification of insulin and FA separately on high performance liquid chromatography (HPLC) for release rate analysis. The FA molecules released from the nanoparticles were quantified along with released insulin to explain particle formation mechanism. Loading of FA and crosslinking salt was varied during nanoparticle synthesis to facilitate the control over the insulin release. Phosphate buffer saline (PBS) was used as the release medium during all release studies because this buffer contains salt composition similar to blood.

2. Materials and methods

2.1. Material used

FA, zinc chloride and sodium hydroxide were procured from SRL Pvt Ltd, India. Hydroxypropyl methylcellulose (HPMC) (molecular formula: $C_{12}H_{20}O_{10}$; molecular weight: 324.2848 Da) was purchased from CDH Pvt Ltd. Two types of insulin, insulin lispro and human recombinant insulin were used in the studies. Insulin lispro (molecular weight 5813.63 g/mol) was purchased from Elli Lilly Co called Humalog Mix 50 which was stored in 5 ml cartridges. The concentration of insulin lispro in each cartridge was 100 IU/ml. Human recombinant insulin was purchased from SAFC Biosciences. Water used in the studies was obtained from Milli-Q water unit of Merck Millipore Life Sciences. All the solvents used during HPLC analysis were of HPLC grade.

2.2. Techniques used

2.2.1. Protein quantified by HPLC measurement

Researchers have used bioassays and UV–vis spectrophotometer for the quantification of protein in the earlier studies [40,41]. However, these techniques were sensitive towards the buffer components specially FA molecules in which the protein was dissolved. Hence, HPLC technique was applied for insulin and FA quantification. A HPLC method was developed just to separate both FA and insulin on UHPLC Dionex Ultimate 3000 (supplied by Thermo Fisher Scientific). A gradient method was developed by mixing different compositions of acetonitrile and water. Acetonitrile (containing 0.1% trifluoroacetic acid (TFA)) and water were mixed in 15/85 (v/v) ratio at the start of this method. The proportion of acetonitrile was gradually increased from 15/85 to 60/40 (v/v) in 7.5 min. The composition was again reduced to 15/85 at 7.5 min and achieved at 8.0 min. The ratio of acetonitrile and water was maintained at 15/85 level till 11.5 min. The column used in this method for separation was C-18 (4.6×250 mm, pore size = 5 μm). Sample detection was performed with the help of the UV detector at 215 nm. The flow rate was maintained at 0.8 ml/minute throughout the detection.

Initially, an aqueous solution of each compound (FA, recombinant insulin, and insulin lispro) in the pure form was quantified by HPLC. Insulin lispro was already dissolved in the buffer for better stability. Thus, identification of the insulin peak among multiple buffer component peaks was necessary. To verify the insulin peak, human recombinant insulin was run using the same method. After identification of insulin peak, FA-insulin mixture samples were studied.

2.2.2. Synthesis of nanoparticles

FA nanoparticles were synthesized using emulsification method [42,43]. First, aqueous FA solution was prepared by dissolving FA in 1 ml of deionised (DI) water by dropwise addition of 1N NaOH while the pH of the solution was kept at 6.5. For encapsulation of insulin, an appropriate volume of aqueous FA solution was mixed with insulin lispro for 15 min to maintain 2, 3, 4, 5 and 6 mg insulin loading. Insulin-loaded FA solution was mixed with 5% HPMC at 1:5 ratio for 6 hours (h). Mixing with HPMC produces nano-domains of insulin loaded under FA nanodroplets. These nano-domains were again mixed with 0.1% $ZnCl_2$ solution in 1:2 ratio for 8 h to obtain nanoformulation crosslinked with zinc cation. Similarly, another set of highly crosslinked nanoparticle formulations was prepared using 1% $ZnCl_2$. Nanoparticle solution (200 μl) was separated for size analysis and the remaining nanoparticle solution was centrifuged at 10,000 rpm for 10 min (Eppendorf centrifuge 5810R). The supernatant was analysed by HPLC to estimate encapsulation

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