Ferrite-Silica-Insulin Nanocomposites (FeSINC) for Glucose Reduction

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ABSTRACT

Proteins find more stable environment upon encapsulation in a silica host, because of polymeric silica frame that grows around the macromolecule and protects them from denaturation. Silica-insulin nanocomposite (SINC) and ferrite coated SINC (FeSINC) prepared by polyelectrolytic condensation of silica precursor on insulin were studied for their ability to control glucose levels. SINC was prepared by acid- base catalysed polymerization in presence of insulin at room temperature by modified Stober's process. FeSINC nanoparticles were prepared by co-precipitation of both ferric and ferrous salts on the bovine insulin loaded silica nanoparticle. The presence of ferrite coating in FeSINC was identified using VSM and quantified from XRF study. The intermolecular interactions in these nanocomposites were studied by FTIR and Raman spectroscopy. An *in vivo* study indicated that FeSINC was biologically active in reducing glucose levels as compared to SINC.

Keywords: silica, ferrite, insulin, nanocomposite.

1 INTRODUCTION

Formation of silica nanosphere in the presence of soft template is well known [1]. It is established that silica coating is chemically inert, biocompatible, hydrophilic and inexpensive. Here, we studied this inexpensive way to encapsulate protein in silica and silica-ferrite hosts as silica-insulin nanocomposite (SINC) and ferrite coated SINC (FeSINC) to avoid denaturation of insulin in the gastric environment. Silica precursor which has a negative charge binds to insulin that is polycationic at pH 2, thereby, forming silica insulin nanocomposite (SINC) with gradual increase in pH to neutral [2].

Insulin silica-ferrite nanocomposite was prepared by acid-base catalysed reaction. The maghemite ferrite coating above the silica prevents the extensive growth of polymeric silica framework thereby favoring the formation of FeSINC nanoparticles instead of large micrometer sized particles. The outer maghemite surface present in FeSINC is biocompatible and can be functionalized by transferrin receptor present in gut line of intestine. In vivo activity and physiochemical properties like microstructure, morphology, magnetic nature, encapsulation efficiency, interaction among the constituent of these SINC, FeSINC were studied using microscopy and spectroscopy. Parameters like pH, ionic strength and amount of protein were found to control the formation of ferrite-silica-insulin nanocomposite. In vivo studies were carried out to check bioactivity of formulation.

2 EXPERIMENTAL

2.1 Materials

Tetra ethyl orthosilicate and bovine insulin were obtained from Sigma-Aldrich. Ferrous chloride, ferrous sulphate, HCl, ammonia and ethanol of analytical grade obtained from local suppliers were used without further purification.

2.2 Synthesis of nanocomposites

SINC, FeSINC were synthesized by 2-step acid-base catalysed method. For SINC, acid catalysed hydrolysis of alkoxysilane precursors was carried out for 10min under stirring in ethanol at a mole ratio of 0.9:17:4 (TEOS: Ethanol: H2O). Later, the reaction mixture transformed into gel with increase in silica network formation as the pH was increased to 7 using ammonia. In order to obtain insulin loaded silica, insulin dissolved in HCl (0.001 N) was added before adding the catalyst. The concentration of insulin in silica sol was 0.4 mg/ml. Polymerisation of silica in presence of insulin was controlled by storing at 0^{o}C. In FeSINC system, the silica framework was controlled by addition of aqueous ferrous sulphate and ferric chloride at a mole ratio of 0.04: 0.08 [3]. The magnetite initially formed over the silica network was transformed into maghemite by decreasing the pH from 7 to 2. The reaction mixture was stirred for 2hr, centrifuged and washed with distilled water. The resultant FeSINC material was lyophilized at -60^{o}C and stored at 4^{o}C.

2.3 Characterisation of nanocomposites

The synthesized materials were characterized by XRF (Model-PW2404, X-ray tube with Rh target, PANAnalytical, Netherlands), TEM (Technai-12, FEI), FTIR (Magna 550, Nicolet Instruments Corporation, USA), confocal Micro Raman spectrometer (Labram HR 800, Horiba Jobin Yvon). The magnetic nature of FeSINC was analysed by vibrating sample magnetometer VSM (Model 7410, Lakeshore USA). BET measurement (Model: ASAP 2020, Micromeritics) was carried to ascertain the porous

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nature of the nanocomposites. Insulin content was measured by HPLC (Waters, Photo diode array detector) using a gradient method.

TEM: Room temperature replica of samples were prepared by placing a drop of sample on a cover slip and allowed to dry at room temperature. Replicas of the sample were made with a 2nm Pt/C and 40nm carbon coating over it and the coated specimen was transferred to sodium hypochlorite and allowed to stand for 1hr to detach the replica from the specimen. Detached replica was further washed with D/W, lifted over bare carbon grid and examined under TEM.

XRF: Silica and ferrite present in FeSINC was quantified by XRF. The ferrite-silica and ferrite-silica-insulin nanocomposite were mixed with methyl cellulose and propanol, dried under IR light, made into pellet and observed by XRF spectrometer.

HPLC: Insulin content of the samples was analysed using HPLC–UV at 210 nm. Gradient elution was performed using 30% acetonitrile and 70% TFA (0.1%) at a flow-rate of 1ml/min and injection volume of 20 µl. Insulin was detected at a retention time of 5.7 min with detection limit of 0.01 mg/ ml. Encapsulation of insulin in SINC and FeSINC was around 55% and 20% respectively.

FTIR: Samples were mixed with KBr and compressed into discs at 20 KN force at room temperature. Bovine insulin powder, SINC, ferrite-silica, FeSINC was analysed.

Confocal Micro Raman Spectroscopy: Samples were focused using Olympus optical microscope with 20 X objective connected with Raman spectrometer. Ar laser, 514.5 nm, 20 mW, 6 A was used. Spectra were recorded as an average of 3 scans with a time span of 30 seconds each.

2.4 Biological activity of SINC and FeSINC in Wistar rats

The biological activity of the insulin encapsulated in SINC and FeSINC was tested in a rat model (Wistar rats, 200+/-25g, n=3) by measuring decrease in blood glucose levels. Animals fasted overnight (16hr) were anesthetized with isoflurane. Experimental animals received a subcutaneous injection of bovine insulin (4 IU/Kg) while the control animals with distilled water and test animals with 25 IU/Kg of insulin encapsulated in desired formulations. Blood samples were withdrawn at 0, 15, 30, 60, 120, 240min after subcutaneous injection. Plasma glucose levels were measured by glucose analyzer.

3 RESULTS AND DISCUSSION

SINC and FeSINC nanocomposites studied here were formed by supersaturation of anionic silanol species above the insulin molecule by polyelectrolytic condensation due to polycationic nature of insulin at pH 2. Initiation of silica growth above the surface of insulin occurs through hydrolysis of silica precursor at lower pH (2-5) that promotes colloid formation by release of alcohol followed by silanol condensation leading to gelation at pH 7. Presence of silica coating around insulin prevents it from denaturation due to pH and enzymes. Silica and SINC nanoparticles prepared were 100-200 nm (Fig. 1, 2) as observed under TEM.

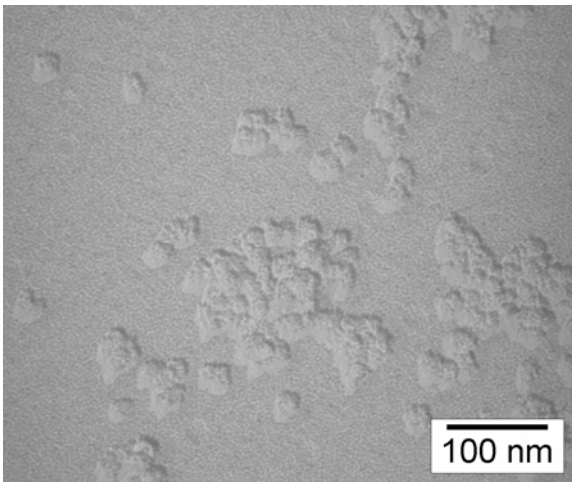

Figure 1: Room temperature replica TEM image of silica nanosphere.

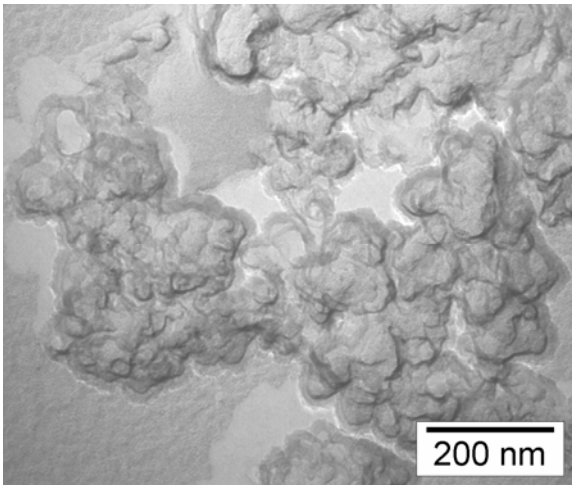

Figure 2: Room temperature replica TEM image of SINC nanocomposite.

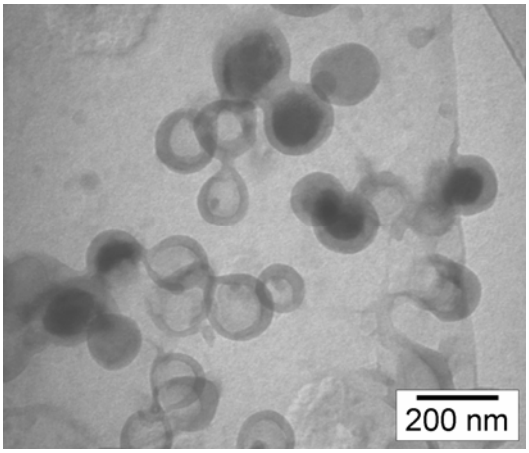

Figure 3: Room temperature replica TEM image of FeSINC nanocomposite.

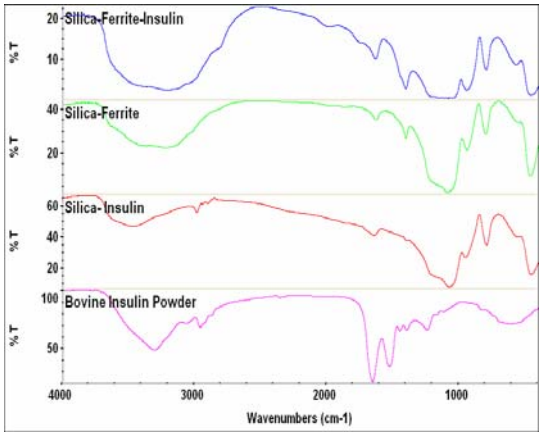

Figure 4: FTIR spectra of bovine insulin powder, SINC, silica-ferrite and FeSINC.

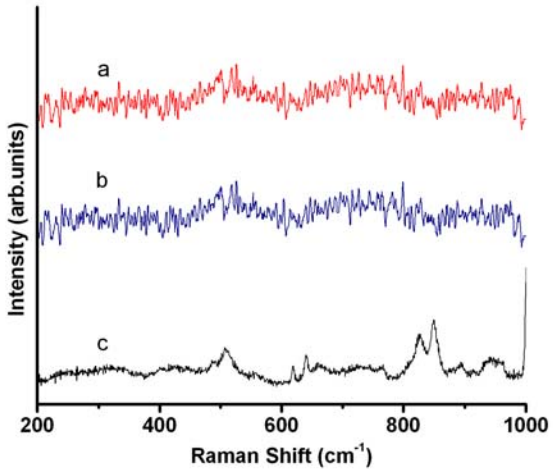

Figure 5: Confocal micro Raman spectra of (a) SINC, (b) FeSINC and (c) bovine insulin powder.

Fused structures of these nanoparticles are due to extended polymeric network of outer silica layer. The maghemite ferrite coating around the silica framework in FeSINC favors well dispersed spherical shape nanoparticles of less than 100 nm by disrupting the silica network with impregnated ferrite particles (Fig. 3). Insulin present in SINC and FeSINC was confirmed from infrared absorption around 3288 cm^{-1} (amide A band), 1644 cm^{-1} (amide I), 1514 cm^{-1} (amide II) and 1236 cm^{-1} (amide III), in comparison with standard bovine insulin by FTIR (Fig. 4). Silica coating in these insulin containing nanocomposite was evident from Si-O stretching at 1080 cm^{-1}, Si-OH at 950 cm^{-1}, Si-O-Si bending at 800 cm^{-1} and Si-O bending at 470 cm^{-1} [4]. The biologically active state of insulin in SINC and FeSINC nanocomposites was identified from Raman shifts due to S-S skeletal bending at 495,505, 517 cm^{-1} and C-S stretching at 668, 680 cm^{-1} by Confocal Micro Raman Spectroscopy in comparison with standard insulin (Fig. 5). Presence of disulphide stretching and amide stretching confirms the chemical stability of insulin encapsulated in SINC and FeSINC [5, 6, and 7].

The XRF results show the maghemite content to be 5% in ferrite silica and 2.5% in FeSINC. Presence of maghemite ferrite in FeSINC was evident from magnetic nature as shown in Fig. 6, 7. Super paramagnetic nature of FeSINC indicated that the ferrite present is less than 50 nm in size. From the magnetization plot, it is apparent that the ferrite content is less in FeSINC which correlates with XRF data. The synthesis protocol involves interaction of insulin and silica at the first stage during which most of the silanol species would have interacted with insulin. In presence of insulin very few ferric and ferrous ion interact with silanol species due to low availability of silanol host, thus leading to a decrease in ferrite content and low magnetic moment of FeSINC.

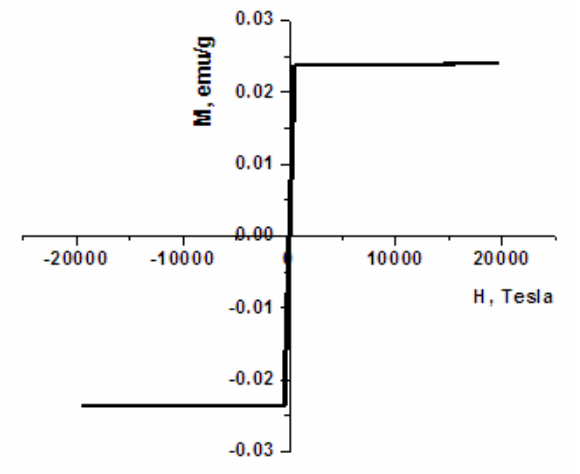

Figure 6: Magnetization of ferrite-silica nanocomposite.

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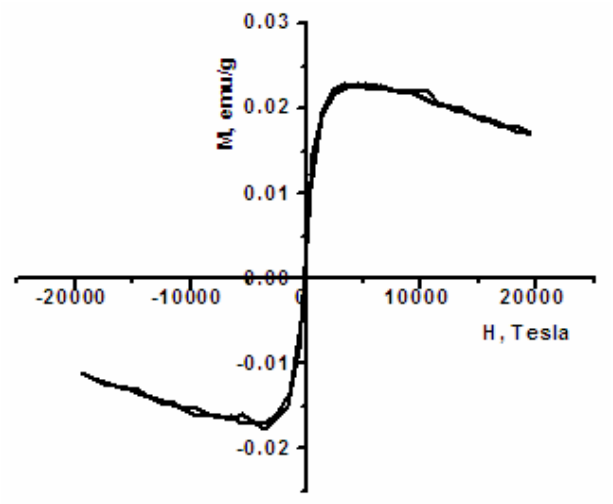

Figure 7: Magnetization of FeSINC nanocomposite.

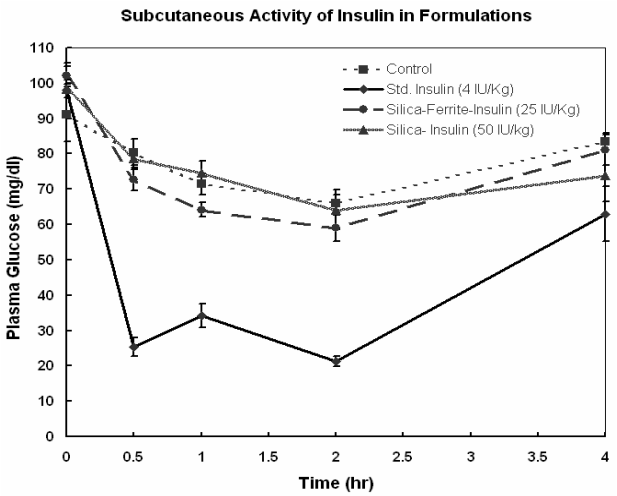

Figure 8: Subcutaneous activity of insulin encapsulated in nanocomposites compared with standard bovine insulin.

BET results indicated that ferrite-silica and FeSINC are porous while SINC is nanocomposites compared with standard bovine insulin.nonporous. Presence of ferrite favored loosely packed silica network by domain segregation thereby forming porous material. Room temperature processed silica xerogels are extensively used as carriers for the controlled release of enzymes, proteins and pharmaceutical substances [8]. Silica coating present in SINC and FESINC prevent it from rapid degradation in the gut lining. Release of insulin from ferrite-silica nanocomposite (FeSINC) may occur through both diffusion and dissolution through pores present in these nanoparticles. Therefore, chemical and structural characteristics of the silica xerogel strongly affect their drug release behavior [9]. The insulin monomer has multiple ionizable groups due to six amino acid residues capable of attaining positive charge and other 10 amino acid residues capable of attaining negative charge [10]. This polyelectrolytic nature of insulin can be attributed for the entrapment of insulin in FeSINC. The decrease in blood glucose level was observed in test animals administered with FeSINC (25 IU/Kg) in contrast with SINC (50 IU/Kg). Although the insulin content was high in SINC, its ineffectiveness in glucose reduction can be due to its compact nature unlike porous in FeSINC. The porous nature of FeSINC helps to reduce the glucose level in blood by releasing the entrapped insulin (Fig. 8). Native state of insulin in FeSINC formulation is confirmed by its bioactivity in corroboration with spectroscopy. Thus the present formulation may be used for oral delivery of insulin.

4 CONCLUSION

Sol-gel derived silica is biocompatible and biodegradable inorganic carrier material. Its bioresorbability occurs by hydrolysis of siloxane bonds in human body and it is excreted via kidneys [11]. The FeSINC formulation described here was found to be effective in reducing glucose levels. Silica framework is a probable host for biological activity of proteins that is otherwise vulnerable for denaturation. The FeSINC formulation may be used for oral delivery of insulin which could enhance the absorption of nanoparticle in intestine through transferrin receptors.

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