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Synthesis of a novel structure for the oral delivery of insulin and the study of its effect on diabetic rats

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ABSTRACT

Common materials used for drug delivery in the body are: liposomes, micelles, polymer capsules, dendrimers, nanoparticles, porous materials, etc. Drug delivery system should be inert, biodegradable, have high biocompatibility and the ability to load large amounts of the drug with known concentration while having a simple and economical sterilizing process. In this study we produced mesoporous silica nanostructures coated with polyamide amine dendrimer that were placed in chitosan-gelatin scaffolds. At every step of the synthesis, the products were identified using different methods, including XRD, FT-IR, SEM, and TGA. The final drug was studied in terms of in vitro & in vivo and MTT toxicity was evaluated.

1. Introduction

Diabetes is a common disease [1]. Today, with the help of innovative drug delivery systems, including nano-carriers like nanocapsules, micelles, mesoporous nanoparticles, dendrimers, and so on [2], diabetics insulin levels are kept constant [3]. MCM-41 is the most efficient member of mesoporous materials. With the generic name M41S, that was synthesized using liquid crystal templating [4] in 1992 in a US oil company [5,6]. We used features of this material such as high specific surface area, a surface suitable for functionalization with various functional groups and large voids [7], to load insulin in this structure. The PAMAM Dendrimer is a kind of Dendrimer with a 3D structure and abundant and regular connections which was discovered in the early 1980s by Tomalia et al. [8]. Dendrimers are synthesis by both divergent and convergent methods. In the divergent method dendrimers grow from outward to the core [9]. However, in the convergent growth, first dendrons are formed and when the growing dendrons are large enough they are attached to a suitable core to form full dendrimers [10]. In both methods, by duplicating the sequence of reactions a material with a higher generation is synthesized [9]. In this study, we used Polyamidoamine dendrimer which was synthesis by the divergent method and was then attached to the surface of silica by urea linkage [11], which leads to further control of insulin release from the mesoporous silica. Furthermore, dendrimers have a pore structure that allowed for the enhancement of the efficiency of drug loading by loading cinnamaldehyde extracted from cinnamon as supplements to reduce blood sugar in diabetic rats. Finally, we used chitosan-gelatin scaffold to carry the designed structure. Due to the unique properties of chitosan (which is derived from the deacetylation of Chitin's crustacean shell and has properties such as biodegradability, antifungal effects and accelerates the healing process [12–14]) and gelatin (which is derived from the hydrolysis of collagen, the most abundant protein in animals such as cattle, pigs and fish [15,16]), the simultaneous use of the two polymers expanded [17]. The positive charges carried by the chitosan amine groups lead to the connection and interaction with polymers such as gelatin [18] and by changing the ratio of gelatin, the mechanical and biological properties of chitosan-gelatin membranes can be improved [19]. In conclusion, the main goal of this study was achieved and drug release was further controlled, even more than before.

2. Material and method

2.1. Material

Insulin was purchased from Exir pharmaceutical company. Streptozotocin injection, chitosan, CTAB surfactant, polyvinyl alcohol and 3-(Triethoxysilyl)propyl isocyanate were purchased from Sigma-Aldrich. Ethylene diamine, methyl acrylate, gelatin, Texas Red and glutaraldehyde solution were obtained from Merck, Germany. All salts and solution used were from Merck, Germany. Distilled and Deionized water was used in this study.

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Abbreviations

MSN	mesoporous silica nanosphere
M41S	mesoporous materials
MCM-41	Mobil Composition of Matter No. 41
PAMAM	polyamidoamine
PPI	*poly(propylene imine)*
CTAB	cetyl trimethylammonium bromide
ICP	propyl isocyanate
DCM	dichloromethane
CS	chitosan
Gel	gelatin
Cin	cinnamaldehyde
PBS	phosphate-buffered saline
NPs	nanoparticles
XRD	X-ray powder diffraction
TGA	thermogravimetric analysis
^{1}HNMR	proton nuclear magnetic resonance
^{13}CNMR	Carbon-13 nuclear magnetic resonance

2.2. Silica extraction from rice bran

To extract the silica content, after washing and filtering the bran was put in the oven to dry at 80 °C for 24 h. The mixture was then boiled with hydrochloric acid 1 M at 80 °C for 1 h. The mixture was rained with deionized water to reach pH 7. The products were dried in an oven at 110 °C for 24 h and then put in a furnace for calcination 4 h at 650 °C.

2.3. Preparation of MCM-41

To synthesize the MCM-41, 5 g silica that was obtained from the previous step was added to 10 g Sodium hydroxide and the mixture was dissolved in 180 ml distilled water and after stirring for 1 h the CTAB surfactant was added. It was then rained into 63.25 g CTAB surfactant that had been dissolved in 150 ml distilled water. To adjust the pH to 11, 21 ml of HCl 5 M was added drop wise to the solution and refluxed or 72 h at 100 °C. The obtained sediment was then washed with lots of distilled water to reach pH 7. Next, the products were dried in an oven at 80 °C for 24 h. It was then put in a furnace to be calcined at 500 °C for 6 h.

2.4. Preparation of MCM-41 loaded by insulin

To prepare the MCM-41 loaded by insulin, 50 mg insulin was added into 0.5 g MCM-41 that was solved in 5 ml methylene chloride solution. The obtained suspension and polyvinyl alcohol solution (%2W/V) was transferred to a rapid stirrer (solution temperature was kept at 0 °C). The resulting emulsions containing insulin-MCM-41 are in a continuous aqueous phase. After filtering, the emulsion was put into the desiccator in order to dry.

2.5. Preparation of PAMAM G2

2.5.1. Preparation of PAMAM G0.5

To prepare the PAMAM G0.5, 5.5 ml ethylene diamine was dissolved in 20 ml methanol and then added to a solution containing 37 ml methyl acrylate and 20 ml methanol. The mixture was vigorously stirred for 30 min at 0 °C, and then allowed to warm to room temperature and stirred for 24 h. Then in order to remove the solvent, the solution was placed on a rotary evaporator under nitrogen atmosphere.

2.5.2. Preparation of PAMAM G1

To prepare the PAMAM G1, 10 g from the output of the previous step was dissolved in 20 ml methanol and stirred at 0 °C. It was then added to a solution of 85 ml ethylene diamine and 100 ml methanol drop by drop. Then the mixture was stirred for 96 h at room temperature and the solution was placed on the rotary evaporator.

2.5.3. Preparation of PAMAM G1.5

To prepare the PAMAM G1.5, 8 g product from the previous step was dissolved in 20 ml methanol and while being stirred at 0 °C, a solution of 13.5 ml methyl acrylate and 20 ml methanol was drop wise added to it. The solution was then allowed to warm to room temperature and stirred at room temperature for 24 h. The solution was then placed on the rotary evaporator under nitrogen atmosphere.

2.5.4. Preparation of PAMAM G2

To preparation the PAMAM G2, when 3 g of the product from the previous step was dissolved in 10 ml methanol and stirred, a solution of 32.5 ml ethylene diamine and 50 ml methanol was drop wise added to it. The mixture was stirred for 96 h at room temperature. Then the solution was placed on the rotary evaporator.

2.6. Preparation of MCM-41 connected PAMAM G2 by urea linkage

To prepare the MCM-41 connected PAMAM G2, 0.25 ml 3-(Triethoxysilyl)propyl isocyanate was added into the solution containing 1 g MCM-41 and 80 ml toluene, and after 20 h the propyl isocyanate was grafted onto the pore surface of the MSN (ICP-MSN). To visualize the reaction, 0.15 g ICP-MSN was added to aqueous ethanol solution of Texas Red (a fluorescent dye, 5 mM), then the mixture was stirred for 20 h. The ethanol solution of 0.11 mM G2-PAMAM was then added to that solution and after 20 h a urea linkage between the amine groups of the Polyamidoamine and propyl isocyanate groups of MSN arose (G2-MSN).

2.7. Extract cinnamaldehyde from cinnamon

To extract cinnamaldehyde from cinnamon, Cinnamon sticks were placed into a mortar and minced to medium pieces. The crushed cinnamon was then added to 100 ml distilled water in a balloon flask with two openings. Then an addition funnel containing 100 ml distilled water was attached to the next opening. The mixture in the round balloon flask was stirred at 100 °C. When the distillation started, a cloudy distilled substance could be seen traveling down from the condenser column. The process was continued until the output was clear. Then the distilled product was transferred to a separatory funnel and then DCM was added to wash what was in the separatory funnel. This process was repeated four more times, using DCM each time. The DCM was washed back into the separatory funnel and dried using a saturated sodium chloride solution and then rained to the flask containing calcium chloride. After 5 min, the mixture was filtered and rained directly into the flask and another distillation was carried out to recover the DCM. When all of the DCM had been removed, the yellow oil remaining was identified as cinnamaldehyde.

2.8. Preparation of G2-MSN loaded by cinnamaldehyde (Cin-G2-MSN)

To prepare the G2-MSN loaded by cinnamaldehyde, 5 mg G2-MSN was added to an aqueous solution of cinnamaldehyde (1 mg/ml) and stirred at room temperature in darkness for 24 h. The solution was then placed into a vacuum desiccator in order to evaporate the solvent.