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Design and Development of Glucose-responsive Nanoparticles for controlled Insulin release

Pooja Arora[1], Anshu Rajpurohit[2], Vijay Kumar Bansal[3], Anuradha Eknath Gavade[4], Pritam Bhagawat Chaudhari[4], Pratiksha Prathamesh Nikam[4], Rajesh Kumar Sharma[5] and Lalita Shashikant Nemade[*6]

[1]Swami Devi Dyal Institute of Pharmacy, Golpura, Barwala, Dist: Panchkula, Haryana (India)
[2]Department of English, Career Point University, Kota, Rajasthan (India)
[3]Lachoo Memorial College of science and Technology, Jodhpur, Rajasthan (India)
[4]Govindrao Nikam College of Pharmacy, Sawarde, Tal: Chiplun, Dist: Ratnagiri: 415606, M.S., (India)
[5]Department of Pharmacognosy, Teerthanker Mahaveer College of Pharmacy, Teerthanker Mahaveer University, Delhi Road, Moradabad-244001 (India)
[6]Department of Pharmaceutics; Govindrao Nikam College of Pharmacy Sawarde, Dist: Ratnagiri: 415606 (India)
***Corresponding author**: Dr. Lalita Shashikant Nemade; Professor, Department of Pharmaceutics; Govindrao Nikam College of Pharmacy Sawarde, Dist: Ratnagiri: 415606 (India)

Abstract

The global burden of diabetes mellitus, characterized by defective insulin secretion or action, necessitates advanced therapeutic strategies. Glucose-responsive insulin (GRI) systems, which autonomously release insulin in response to hyperglycemia, represent a paradigm shift toward a closed-loop therapy at the molecular level. Two boronic acid-chitosan conjugates were synthesized using 4-formylphenylboronic acid (FPBA) and 2-formyl-3-thienylboronic acid (FTBA). Conjugation was confirmed via Fourier transform infrared spectroscopy (FTIR) and differential scanning calorimetry (DSC). Glucose adsorption capacity and selectivity were quantified using high-performance liquid chromatography (HPLC) with a hexokinase assay and a curcumin-based fluorescent displacement assay, respectively. FTIR and DSC analyses confirmed successful conjugation, revealing structural changes and altered thermal properties. Glucose adsorption studies demonstrated that functionalization with boronic acid imparted glucose sensitivity. A 1:1 molar ratio of boronic acid to chitosan was identified as optimal for maximum glucose binding, beyond which increased

[1]Director, [2,6]Professors, [3,4,5]Associate Professors

crystallinity and steric hindrance reduced adsorption. A key finding was that FTBA-chitosan conjugates exhibited a statistically significant ($p<0.025$) higher glucose adsorption capacity than FPBA-chitosan conjugates, attributed to supplemental stabilizing C-H•••π interactions facilitated by the thienyl ring. Novel boronic acid-chitosan conjugates were successfully synthesized and characterized.These findings highlight the critical role of boronic acid moiety structure in designing efficient glucose-sensing materials. The optimized 1:1 conjugates present a promising foundation for the subsequent development of a fully autonomous, glucose-responsive insulin delivery system.

Key words : Glucose-responsive insulin; Smart insulin delivery; Boronic acid; Phenylboronic acid (FPBA); Thienylboronic acid (FTBA); Conjugate; Glucose adsorption.

The Global Burden of Diabetes Mellitus :

Diabetes mellitus represents one of the most significant and escalating public health challenges of the 21st century, a non-communicable pandemic with profound human, economic, and societal costs. Characterized by chronic hyperglycemia resulting from the body's inability to produce or effectively use insulin, diabetes is a complex metabolic disorder with two primary forms. Type 1 diabetes (T1D) is an autoimmune condition wherein the body's immune system mistakenly attacks and destroys the insulin-producing beta cells in the pancreas, necessitating lifelong exogenous insulin administration for survival. Type 2 diabetes (T2D), which accounts for the vast majority (over 90%) of cases worldwide, is characterized by insulin resistance, where the body's cells do not respond effectively to insulin, often coupled with a progressive decline in insulin secretion. The global prevalence of diabetes has reached alarming proportions. According to the International Diabetes Federation (IDF), approximately 537 million adults were living with diabetes in 2021, a figure projected to rise to 643 million by 2030 and a staggering 783 million by 2045. This rise is inextricably linked to factors such as aging populations, urbanization, sedentary lifestyles, and the increasing prevalence of obesity.[1-4]

Limitations of Conventional Insulin Therapy :

Since insulin's discovery in 1921, exogenous insulin has been vital for managing type 1 and advanced type 2 diabetes. However, conventional methods like multiple daily injections (MDI) or insulin pumps (CSII) are imperfect substitutes for natural pancreatic function. Many users struggle to reach glycemic targets, and fear of hypoglycemia often leads them to maintain higher glucose levels, increasing long-term complications. This constant challenge between avoiding low blood sugar and preventing high levels underscores the limitations of current therapies. These shortcomings have driven the search for more automated and physiological insulin delivery

systems.[5-8]

Fundamentals of Stimuli-Responsive Drug Delivery :

Stimuli-responsive, or "smart", drug delivery systems are engineered to release their therapeutic payload in response to specific physiological or external triggers. For diabetes, the ideal internal stimulus is the fluctuating concentration of glucose itself, paving the way for a self-regulating, closed-loop insulin delivery system that mimics a healthy pancreas.[9-12]

Mechanisms of Glucose-Responsiveness:

Several approaches are used to achieve glucose-responsive insulin release. Glucose Oxidase (GOx)-based systems use the enzyme GOx to oxidize glucose into gluconic acid, lowering pH and triggering insulin release from pH-sensitive polymers. Concanavalin A (Con A)-based systems rely on competitive binding between glucose and glycosylated insulin, where high glucose levels displace insulin for release. Phenylboronic Acid (PBA)-based systems use reversible binding with glucose's diols, causing changes in polymer properties that release insulin at high glucose levels. Comparatively, GOx systems face issues with oxygen dependence and toxicity, Con A systems may cause immune reactions, while PBA systems offer better stability, tunability, and clinical potential.[13-15]

Low molecular weight chitosan, 4-formylphenylboronic acid, 2-formyl-3-thienylboronic acid, sodium borohydride, a glucose hexokinase assay kit, and curcumin were sourced from Sigma-Aldrich. Acetic acid, methanol, acetonitrile, fructose, and glucose were obtained from Thermo Fisher Scientific. All other chemicals were reagent grade.

Synthesis and purification of boronic acid-chitosan conjugates :

Fifty milligrams of chitosan dissolved in 1% acetic acid was mixed with FPBA and FTBA equivalents in methanol. After three hours, sodium borohydride was added. Post-reaction, quenching with NaOH precipitated the product, which was centrifuged, washed, lyophilized, and stored at 2°C.

FTIR & DSC Study :

Fourier transform infrared spectroscopy (FTIR) analyses were performed on a Perkin Elmer spectrometer. Freeze-dried conjugates were mixed with KBr (1:99), pressed into discs, and scanned from 2000 to 400 cm^{-1}. For differential scanning calorimetry (DSC), ~5 mg samples were heated from 25°C to 350°C at 10°C/min under nitrogen.

Absorption of Glucose :

Glucose adsorption was analyzed via HPLC. Conjugates (10 mg) were incubated with 1 mL of 5 mg/mL glucose solution (pH 5.5) at 37°C for 1 hour. After centrifugation, the glucose remaining in the supernatant was quantified. An indirect hexokinase assay was used, where glucose is enzymatically converted, reducing NAD+ to NADH, measured at 340 nm. The adsorbed glucose was calculated from the difference between the initial and supernatant concentrations.

Boronic acid conjugates for glucose vs fructose :

An HPLC method using curcumin as a fluorescent dye was employed to investigate the selectivity of the boronic acid conjugates for glucose and fructose. This assay is based on the competitive displacement of curcumin from the boronic acid binding site by diols, as the formation of the rosocyanine complex is reversible. The relative affinities for each sugar were determined by measuring this displacement.

Statistical Analysis : A simple two-tailed t-test with a 95% confidence interval was used to determine statistical significance where applicable.

Characterization of conjugates :

The conjugation of boronic acids (FPBA and FTBA) to chitosan was confirmed through FTIR analysis. Key evidence includes the disappearance of the primary amine peak at ~1590 cm^{-1}, indicating bonding at that site, and the appearance of a new peak at ~830 cm^{-1}, characteristic of a para-substituted benzene ring from the FPBA, confirming successful functionalization.

For the FTBA-chitosan conjugate, FTIR analysis confirmed successful conjugation, evidenced by the disappearance of the primary amine peak at ~1590 cm^{-1}. However, the absence of a peak at 830 cm^{-1} distinguished it from the FPBA conjugate, as FTBA lacks a benzene ring. DSC thermograms showed the conjugates had a lower decomposition temperature than pure chitosan, supporting the hypothesis of progressive primary amine consumption during conjugation.

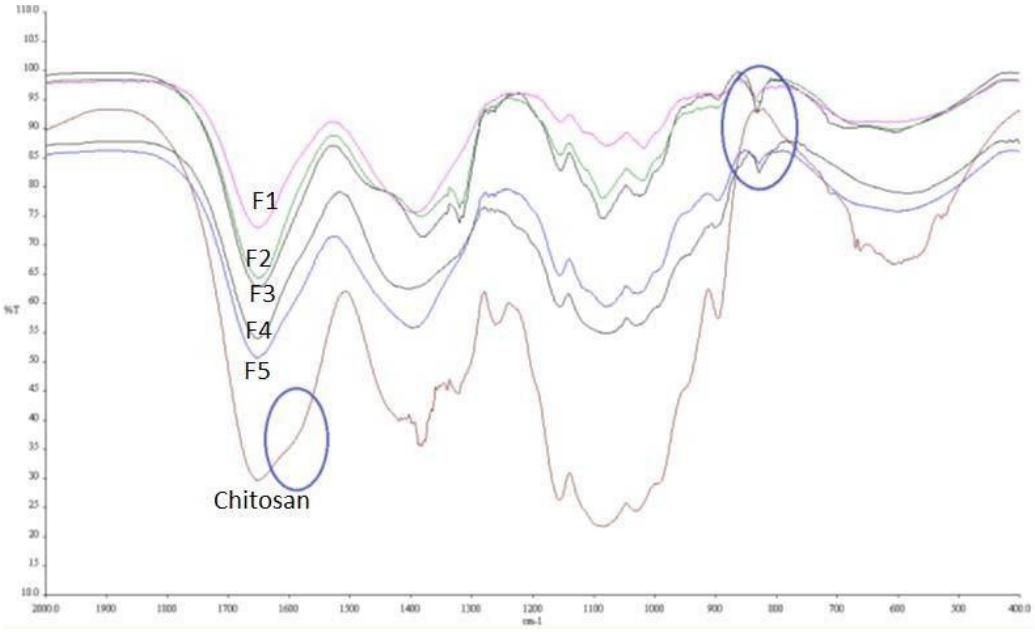


Fig. 1. FTIR spectra of pure chitosan and FPBA-chitosan

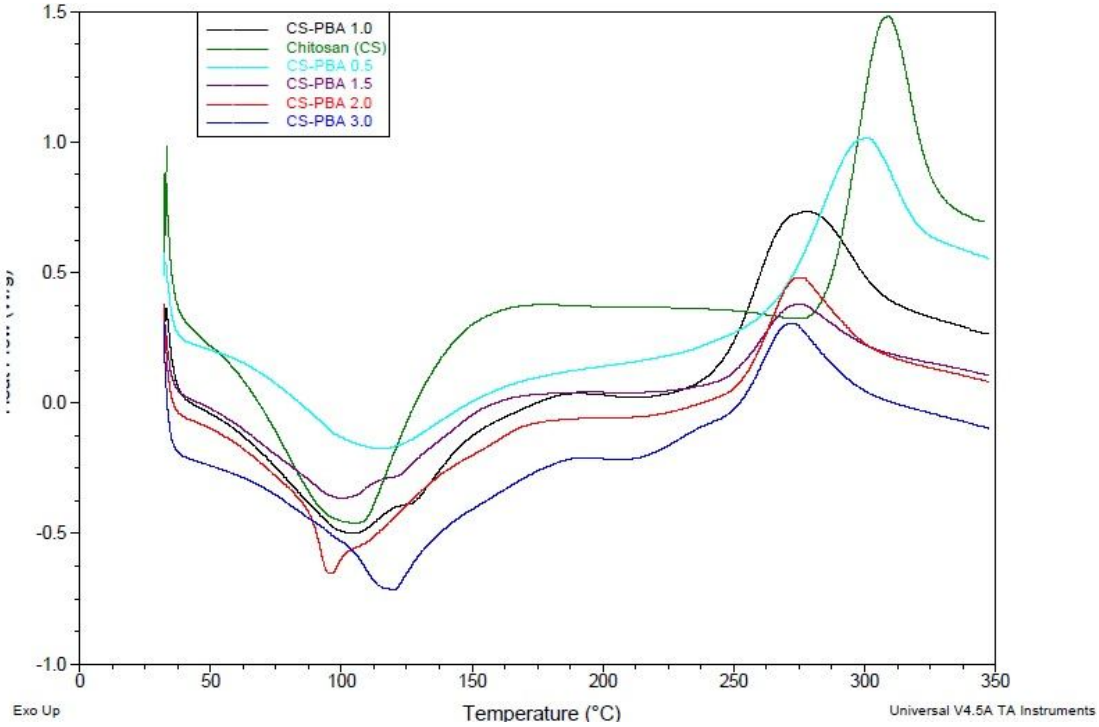
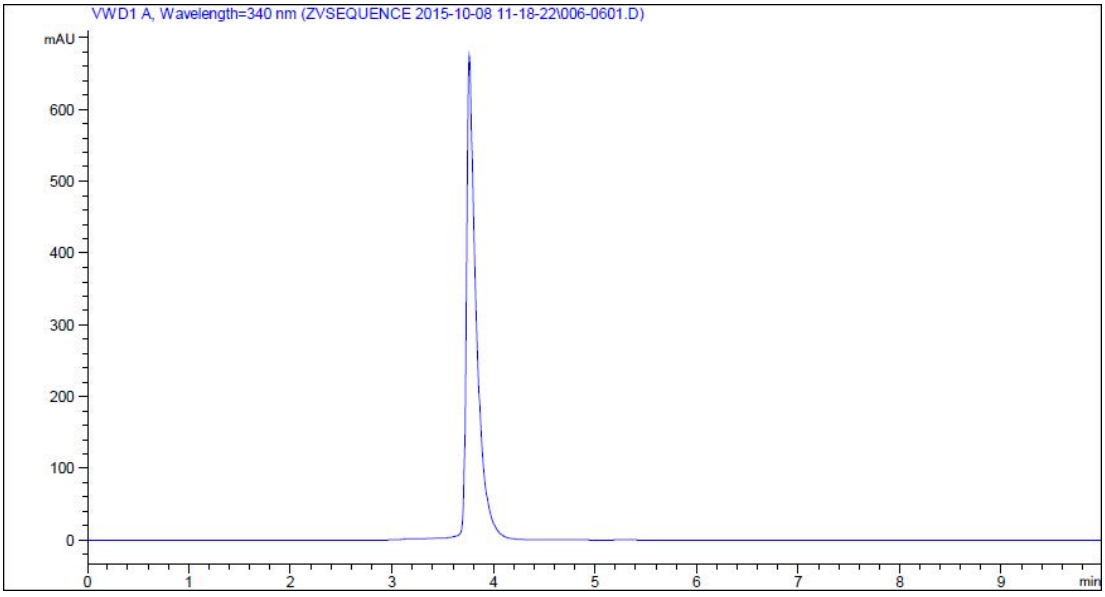


Fig. 2. DSC thermograms of pure chitosan and FPBA-chitosan



(a)

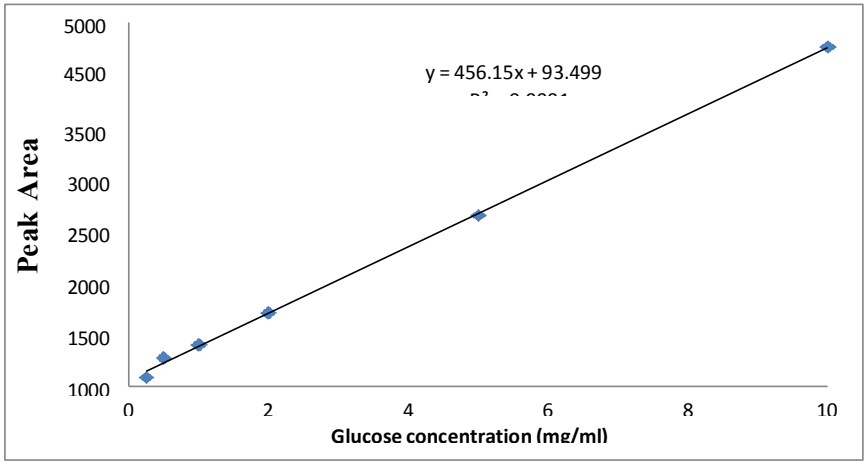


Fig. 3 (a) Chromatogram of NADH from glucose standard (10mg/ml) and (b) Calibration curve of glucose standards

Finally, HPLC analysis using the hexokinase assay produced a highly linear calibration curve ($R^2 \approx 1$), validating the method for quantifying glucose adsorption and indirectly confirming successful boronic acid functionalization.

The glucose adsorption results confirm successful conjugation, as only the boronic acid-functionalized chitosan adsorbed glucose. Adsorption increased up to a 1:1 molar ratio of boronic acid to chitosan, indicating this is the optimum for maximum binding capacity. Beyond this ratio, adsorption decreased despite higher boronic acid loading. This is attributed to increased conjugate crystallinity and polymer backbone rigidity, which creates steric hindrance that prevents the multivalent binding required for effective interaction with glucose's spatially separated hydroxyl groups.

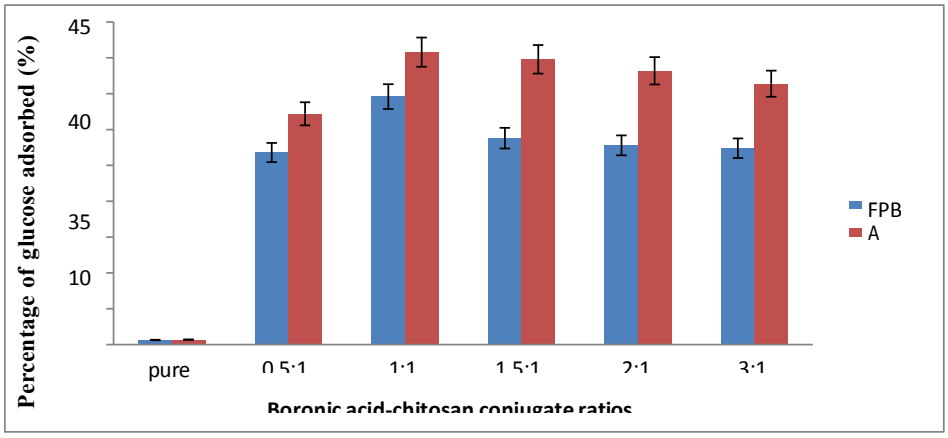


Fig. 4. Glucose adsorption of FPBA and FTBA conjugates

A significant finding ($p<0.025$) was that all FTBA-chitosan conjugates demonstrated higher glucose adsorption than their FPBA counterparts. This superior performance is attributed to the molecular structure of the thienyl ring in FTBA. It is postulated that the proximity of the thienyl ring and the chitosan's cyclohexane rings to the bound glucose facilitates additional stabilizing C-H•••π interactions. These supplemental non-covalent forces are not as feasible with the phenyl ring of FPBA, leading to the slightly higher affinity and greater adsorption capacity observed for the FTBA conjugates.

Two boronic acid-chitosan conjugates (FPBA and FTBA) were successfully synthesized and characterized. Both demonstrated glucose sensitivity under biologically relevant conditions. A key finding was that FTBA conjugates exhibited a significantly higher glucose adsorption capacity than their FPBA counterparts, attributed to advantageous structural interactions. For both types, a 1:1 molar ratio of boronic acid to chitosan was identified as the optimum for maximum glucose binding, as higher ratios induced steric hindrance. Based on these results, the 1:1 conjugates were selected for subsequent insulin nanoparticle formulation and release studies.

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