International Journal of Biological Macromolecules 83 (2016) 376–384

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

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

Functional properties of chitosan built nanohydrogel with enhanced glucose-sensitivity

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ARTICLE INFO

Article history:
Received 8 September 2015
Received in revised form 29 October 2015
Accepted 12 November 2015
Available online 17 November 2015

Keywords:
Nanohydrogel
pH
Glucose sensitivity
Zeta potential
Drug release profile

ABSTRACT

A new approach to design multifunctional chitosan based nanohydrogel with enhanced glucose sensitivity, stability, drug loading and release profile are reported. Two approaches were followed for functionalization of chitosan based nanohydrogel with 3-APBA via EDC and 3-APTES. The effective functionalization, structure and morphology of Chitosan based IPN respectively were confirmed by FTIR, SEM and AFM. At physiological conditions, the glucose-induced volume phase transition and release profile of the model drug Alizarin Red with 1,2-diol structure (comparative to insulin as a drug as well as a dye for bio separation) were studied at various glucose concentrations, pH and ionic strengths. The results suggested a new concept for diabetes treatment and diols sensitivity in view of their potential hi-tech applications in self-regulated on–off response to the treatment (drug delivery and bio separation concurrently).

1. Introduction

Cell metabolism is the key objective of sensor technology to monitor cell responses to therapeutics and antibiotics. Glucose sensitivity of hydrogel containing glucose-oxidase being a catabolic enzyme with poor stability is reported to reduce the substrate level, hence affecting the cell metabolism [3,11,28]. Thus the ability of PBA (Phenylboronic acid), a synthetic ligand for binding glucose in aqueous medium with better recognition to its counterpart in small volume bioreactors validated its application as moiety in glucose sensor technology. The only problem with PBA is its detection of glucose in alkaline media due to higher pK_a ~8.8 values. Also the existence of charged PBA in tetrahedral state at pH values above the pK_a ~8.8 which bind the diols more readily as compared to uncharged trigonal planar configuration which does not readily complex with cis-diols is a challenge to monitor glucose at physiological conditions. So functionalization of PBA with groups to work at physiological pH is one of the key success for future development [9,19]. Thus incorporation of amino group to PBA leads to work at physiological pH. Infact amine group stabilize the complex formed between PBA and diols. One way of explanation is Lewis acid–base interaction which occurs between electron deficient boron in PBA and electron rich nitrogen in the amine group. Also stronger interaction leads to better solvolysis and much faster time response ($t_{1/2}$ < 5 min) [8,21].

The challenges to find a hydrogel system which would respond only to glucose variations at physiological pH, pharmacokinetics similar to normal pancreatic activity, biocompatibility without any in vivo toxicity and possibly no long term side effect.

Chitosan, the second most abundant natural biopolymer after cellulose, is a linear polysaccharide composed of randomly distributed- (1-4)-linked D-glucosamine and N-acetyl-D-glucosamine units. Actually ionic interactions occur between cationic amino groups of chitosan and negatively charged biomolecules or anion of metals like Pt (II), Pd (II) or Mo (IV) in extreme sensitivity. Also the interchains interaction include H-bonding between ionic molecules and chitosan OH groups or between deacetylated chitosan. Charge density of the small molecules and chitosan is influenced by environmental pH and the material pK_a values [2,4].

The use of (3-dimethylaminopropyl)-3-ethyl carbodimide hydrochloride commonly known as EDC as coupling agent in pharmaceutical formulations which readily hydrolyze and form urea which can be removed from the body by water extraction, is recently employed. We compared its effect with another coupling agent known as 3-aminopropyltriethoxy silane which is the most

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http://dx.doi.org/10.1016/j.ijbiomac.2015.11.040
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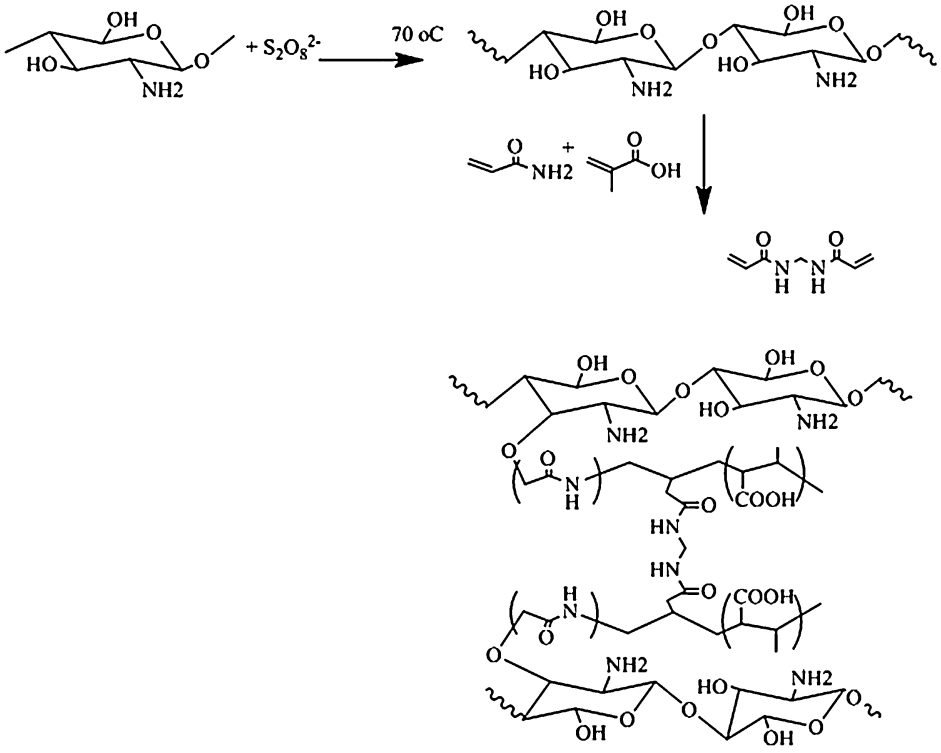

Fig. 1. Proposed mechanism for synthesis of IPN of Chitosan based hydrogels.

used coupling agent in drug delivery systems [16,18]. 3-APTES containing propyl group and a distal amine group for biomolecule attachment. An alkyl salinization is the surface chemistry applied for biosensing, interferometric detection of protein, DNA and also used for the immobilization of bio-molecules for a variety of biomedical applications to build bio-recognition interfaces [17,24]. To compare the properties of both the coupling agents, the effect of surface chemistry-induced charge on the particle uptake was explored in this work. It was observed that due to silane coupling, the PBA were capable of rapidly internalizing with glucose molecules. These finding suggests the finest design of drug delivery vehicles in account to find presentation of glucose molecules.

Fundamental physicochemical and electrokinetic investigations are of utmost importance in order to understand the pH dependency toward glucose sensitivity of the chitosan based nanohydrogel which govern the interaction with the biomolecules (glucose, enzymes, proteins, DNA and dyes). So how and why the changes occur in the hydrogel system with the environment (glucose, pH, ionic strength) depends upon the physical and chemical properties of the system [20,25,30]. Several workers reported the chitosan based hydrogel for drug delivery focusing on the macroscopic films, crosslinkers, microspheres and the reaction conditions but still the effect of internal and external parameters needed to be addressed. Our efforts showed enhanced results with desirable properties by introducing a new coupling agent into such systems, which tuned the internal and external properties. The investigation of physicochemical, electrokinetic parameters with a model drug loading Vs release profile simultaneously by the new coupling agent may attracts the scientist for future drug delivery with pre-determined physical and chemical properties.

Chitosan-poly (acrylamide-co-methacrylic acid) IPNs were prepared by free radical co-polymerization using methylenebisacrylamide and ammonium peroxysulphate as crosslinker and initiator respectively. The effect of pH, glucose and ionic strength on physicochemical and electrokinetic investigations in terms of swelling, zeta potential, conductance and electrophoric mobility were studied in detail. The model drug Alizarin Red (comparative to insulin as a drug as well as a dye for bio separation) was used to study the loading and release profile for the hydrogel in terms of absorbance by using UV–vis-Spectroscopy. Consequently the pronounced effect of 3-APTES coupling was determined with enhanced sensitivity toward diols.

2. Experimental

2.1. Materials

Chitosan ($M_w \approx 160{,}000$ g/mol, degree of deacetylation DD ≈ 90%) N,N-methylenebisacrylamide (MBA) were obtained from Acros (Geel, Belgium), while all other chemicals were purchased from Aldrich (St. Louis, MO, USA). Methacrylic acid (MAA) which was purified by distillation at reduced pressure to remove hydroquinone inhibitor. 3-aminophenylboronic acid (3-APBA), N-ethyl-carbodimide hydrochloride (EDC), D(+)-Glucose, 3-aminopropyltriethoxy silane (3-APTES) and all buffer solutions were used as received without purification. Deionized distilled water (DDH_2O) followed by filtration through a 0.2 μm filter to remove any dust was used for all solution preparation, polymerization, dialysis and analysis steps.

2.2. Synthesis of chitosan-poly (acrylamide-co-methacrylic acid) hydrogel

Chitosan-poly (acrylamide-co-methacrylic acid) hydrogels were synthesized by free radical co-polymerization. Initially chitosan (500 mg) was dissolved in 80 mL (DDH_2O + 0.35 mL acetic acid) with constant stirring for 06 h at room temperature, followed by the addition of AAm, MAA in a three neck round bottom flask equipped with N_2 inlet and condenser with constant stirring for 10 h at room temperature. After 16 h stirring under N_2 purging at room temperature the crosslinker (MBA dissolved in 10 mL of DDH_2O was added to the solution drop-wise and temperature was