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Chitosan nanoparticles as a biocompatible and efficient nanowagon for benzyl isothiocyanate

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ABSTRACT

A plethora of evidences support the health benefits of a sulfur containing compound called Benzyl Isothiocyanate. However, its therapeutic application is limited due to its low solubility, poor stability and inadequate bioavailability. The problem has been worked upon and resolved by the synthesis of biodegradable nanoparticles using chitosan as the controlled delivery nanowagon. The prepared nanoparticles have been characterized using UV–visible absorption spectroscopy, IR spectroscopy, XRD, TGA, TEM and FE-SEM. Results reveal that loading of benzyl isothiocyanate into chitosan nanoparticles increases its solubility and stability. The maximum encapsulation efficiency was obtained to be 64.68 ± 4.7% with slow and sustained release of 77.78% in 144 h at pH 5.5. Clear enhancement in the stability of benzyl isothiocyanate that is sensitive to ultraviolet light has been showcased after its encompassment in the cationic polymer. Further the biosafety of the fabricated system has been demonstrated by haemolysis and its interaction with biomolecules. The antimicrobial activity connotes that the prepared nanoparticles can act as a useful and safe carrier for the loading of benzyl isothiocyanate making it a promising formulation for biological applications in future.

1. Introduction

The medicinal importance of organosulfur compounds- Isothiocyanates (ITCs) dates back to holy ancient writings. ITC-rich herbs were used in cooking, in pickle preservation, as tooth cleaning agents and in massage oils as relaxing and antimicrobial agents. ITCs are found in abundance in a wide variety of cruciferous vegetables like broccoli, garden cress, Alliariapetiolata etc. [1]. One of the important members of ITC family is Benzyl Isothiocyanate (BITC) (Scheme S1, Supporting information). This bioactive has remarkable bacteriostatic and bactericidal properties. It has also demonstrated its potential as an anthelmintic and vermifungal agent [2] and exhibits susceptibility on a variety of pathogenic microorganisms at low concentrations, making it a promising antimicrobial candidate [3]. It also showcases the capability of causing functional aberration in isolated uterus [2]. Epidemiological studies by Li et al. [4] suggested that the chemicals contained in cruciferous vegetables can act as efficient antimicrobials and serve as safer and more effective alternatives to conventional antibiotics [3].

Till date, the therapeutic properties of BITC have not been exploited to its full potential since it readily forms *N*-acetyl-S-(N-benzylthiocarbamoy1)-L-cysteine metabolite and on the average 53.7% of the dose of BITC gets excreted as cysteine metabolite by the renal route [5]. The embryonic use of BITC has also been hindered by its pungent smell, poor bioavailability, easy degradation and low aqueous solubility. Besides, it is strongly volatile and the scientific community for the present is therefore, bereft of any mechanism to fix its appropriate dose.

Researchers are at work to completely attain or at least improve the stability of ITCs with the identification of suitable carriers. Ohta et al. [6] reported cyclodextrins (CD) as plausible carriers for such compounds. The annexed action of ITCs and CDs has been reported to be helpful in inhibiting the decomposition of ITCs in aqueous solution [7–9]. Besides, efforts have also been made by Qhattal et al. [10] for formulating Nano emulsion with improved solubility and enhanced activity of BITC. But the reported carriers suffered from the drawbacks of burst release and size control, so size controlled nano-module have been fabricated for controlled release of BITC.

Biodegradable polymers like chitosan (Chi) have showcased promising results as nano-delivery modules owing to its ability to increase the drug stability, improve residence time and reduce drug degradation [11]. Chitosan, a natural nontoxic biopolymer, has exclusive structure, revealing wide ranging physicochemical properties with striking

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biological applications but till now there is no report unleashing the untamed potential of Chi for the delivery of BITC [12]. Non-toxic Chi has been documented as a propitious polymer for drug delivery due to the ability to remain in blood stream for a relatively longer period of time facilitating the bio-actives to reach target sites. It also evinces antibacterial activity in an acidic medium owing to its poor solubility above pH 6.5 [13].

Therefore, an attempt has been made in the present study to prepare nano formulation of BITC using biodegradable biopolymer chitosan through an alternative methodology. The study focuses on enhancing the solubility and stability of BITC with improved bioavailability and greater retention time. The prepared nanoparticles have been completely characterized using UV–visible absorption spectroscopy, Infrared spectroscopy, X-ray diffraction (XRD), Thermo- gravimetric analysis (TGA), Transmission electron microscopy (TEM) and Field emission scanning electron microscopy (FE-SEM). An attempt has also been made to establish a link between physicochemical properties and physiological parameters. Interaction of nanoparticles with model biomolecules (biotin and riboflavin) has been done to confirm the bioreactivity and thermodynamic parameters have been evaluated. The controlled release studies have been conducted at pH 5.5 and 7.2. The haemo-compatibility and bio safety of the prepared BITC Chi NPs have also been analyzed. Furthermore, the biological potential has been explored through antimicrobial activity.

2. Materials and methods

2.1. Chemicals and reagents

Low molecular weight Chitosan (≈50 kDa) with deacetylation degree of (75–85%), and sodium tripolyphosphate (TPP), benzyl isothiocyanate (BITC), acetic acid, biotin (99%), riboflavin (≥99%) and Tween-80 (purity > 99%), were purchased from Sigma-Aldrich. All the chemicals were used as such for the present study without further purification. Triple distilled water with conductivity <3 μS cm^{-1} was used in all experiments. Chemical structures of Chi and BITC are shown (Scheme S1, Supporting information).

2.2. Methods of preparation

2.2.1. Synthesis of Chi and BITC Chi NPs

In the present work, Chi NPs and BITC Chi NPs were prepared by employing emulsion ionic gelation technique with modification [14]. The Chi NPs were synthesized by two-step method i.e. oil-in-water (o/w) emulsification followed by ionic gelation of Chi with TPP (Scheme 1). Chi solution (1.2%, w/v, 30 ml) was prepared by agitating Chi powder in aqueous acetic acid solution (1% v/v) with ultra turrax homogenizer at 4000 rpm followed by water bath sonication for 30 min. Tween 80 was added as a stabilizer to Chi solution and the mixture was stirred for 120 min at 300 rpm to obtain a homogeneous solution. 0.5% (w/v, 30 ml) solution was slowly added dropwise with constant stirring till the formation of nanoparticles. Solution was centrifuged at >7500 rpm and the supernatant containing the Chi NPs was harvested and the mean diameter was measured by light scattering.

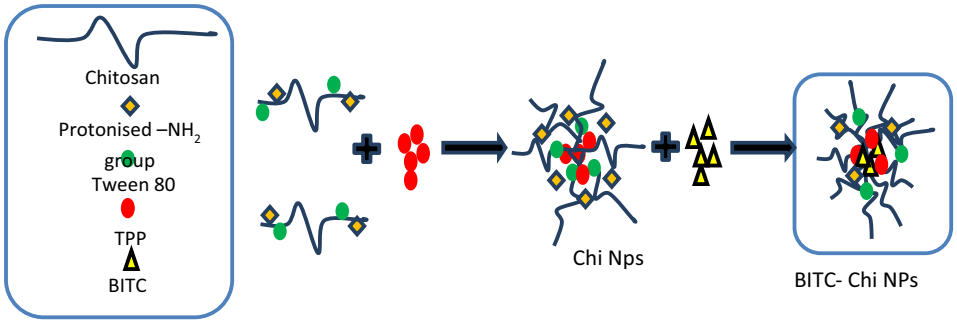
BITC Chi NPs were prepared by the same method. BITC was dissolved in ethanol and added to the prepared Chi solution and stirred for 20 min. 0.5% TPP solution was then added dropwise with constant stirring. Solution was centrifuged at >7500 rpm and the supernatant were harvested and lyophilized to obtain the BITC Chi NPs. The prepared NPs were characterized with available techniques such as DLS, TEM, FE-SEM, XRD and TGA, to estimate parameters like size, physical state and morphology.

2.3. Characterizations

2.3.1. Physicochemical characterizations

Abbemat 500 refractometer from Anton Paar, Kruss K20 tensiometer (Germany) with sterile platinum ring and Brookfield DV-II+ ProExtra Rheometer was procured to obtain the refractive indices, surface tension and viscosity of the synthesized nano-formulations, respectively. Each analysis was done thrice at 25 °C, 30 °C and 37 °C. The ring method was adopted for surface tension measurements while viscosity of the prepared NPs was discerned without dilution using HB-1 spindle. The spindle speed was adjusted to 60 rpm and 5 ml solution was used for each measurement with a wait time of 15 min for each operation. FTIR spectra were recorded with thermally-controlled diode laser in the spectral region of 4000–500 cm^{-1} using thermo scientific, Nicolet iS50 FT-IR. UV–visible absorption spectra were acquired in the spectral range of 200–500 nm using Dynamica Halo DB-20 UV–vis Double Beam Spectrophotometer with wavelength accuracy of ±0.3 nm, noise level 0.0003Abs (500 nm) and stray light ≤0.005%. Thermal behaviour was investigated using SDT-Q-600 (TA instruments New Castle, DE) with flow rate of nitrogen, 100.0 ml min^{-1}. Powder X ray diffraction (XRD) studies were carried out using Brukner D8 Advance X-ray diffractomer equipped with Cu-Kα radiation source (λ = 1.541 A) and step size of 0.015° under the accelerating voltage of 40 kV and 25 mA.

The surface morphology was analyzed using Hitachi-SU8010 field emission Scanning Electron Microscopy (FE-SEM) operating at a voltage of 15 kV and energy dispersion X-ray spectroscopy analysis were carried out with Brooker-Xflash. The particle size evaluations were conducted using Malvern Zeta Nano S 90 (Malvern Instruments, Malvern, UK). Intensity-weighted mean particle size (*Z*-average, nm ± S.D.) using the Stokes Einstein equation was calculated assuming spherical dropletssize by taking average of five measurements involving 5 runs each. Zeta potential was measured using Malvern zeta potential (Nano Sight NS500) at 25 °C. Transmission Electron Microscopy (TEM) analysis was done to determine the shape and size of the dispersed phase. The samples were analyzed using Hitachi (H-7500), equipped with CCD



Scheme 1. Synthesis of Chi NPs and BITC Chi NPs using Ionic gelation method.