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Size-dependent optical properties of bio-compatible ZnS:Mn nanocrystals and their application in the immobilisation of trypsin

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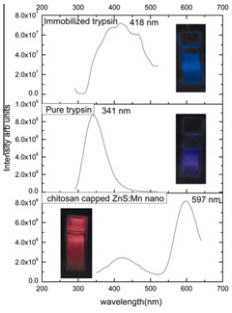
HIGHLIGHTS

- Size dependent optical properties of biocompatible ZnS:Mn nanocrystals were studied.
- Trypsin was immobilised on chitosan capped ZnS:Mn nanocrystals using glutaraldehyde.
- Immobilised trypsin shows increase in its activity compared to free enzyme.
- This work offers prospects of applications in therapeutic and diagnostic fields.

GRAPHICAL ABSTRACT

Upon immobilisation with chitosan capped ZnS:Mn nanoparticle, the activity of trypsin increases, and it becomes stable and active. The conjugation of chitosan capped ZnS:Mn nanoparticle (orange emission) with trypsin can be confirmed from the PL emission shift from violet (pure trypsin) to blue (immobilised trypsin). This work offers prospects of applications of immobilised trypsin in therapeutic and diagnostic fields.



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ABSTRACT

Chitosan capped zinc sulphide nanocrystals doped with manganese (ZnS:Mn) have been synthesised by chemical capping co-precipitation method and structurally characterised by XRD, TEM and EDXS techniques. The dependence of optical properties on the size of these bio-compatible ZnS:Mn nanocrystals was investigated by UV/Vis and photoluminescence (PL) spectroscopic techniques in aqueous solvents. A variation in molar concentration of the precursor, sodium sulphide, from 0.125 to 0.01 mol L^{-1} is accompanied by a decrease in particle size. The excitonic peak in the UV/Vis spectra is found to be blue shifted with a decrease in size of the nanocrystals due to confinement effects. In the present study, trypsin was immobilised onto ZnS:Mn nanocrystals using glutaraldehyde (GA) as cross-linker, which was confirmed by photoluminescence (PL) and Fourier transform infrared (FTIR) spectroscopic studies. Results indicate that the activity of trypsin, immobilised onto chitosan modified nanocrystals, has improved upon cross-linking, which suggests that the immobilised trypsin has become more stable and active. This work highlights the prospects of potential applications of immobilised trypsin in therapeutic and diagnostic fields.

Introduction

Advancements in the synthesis of water-dispersible nanometer sized fluorescent semiconductor materials and the understanding

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of their optical properties have accelerated their practical applications in several fields. These bio-compatible nanocrystals have received much attention because of their potential biomedical applications in imaging, drug targeting and delivery. The conjugation of tiny nanoparticles with specific biomolecules allows researchers to target the desired location, reduce overall toxicity, and enhance the efficiency of the imaging probes [1]. Rational modification in the composition and structure of the nanoparticles, using safer materials, increases the prospects of their usefulness in protein delivery and transport [2].

Major research issues in protein delivery include the stabilization of proteins in delivery devices and the design of appropriate protein carriers [2]. Immobilisation is an important step in commercial and fundamental enzymology for repetitive economic utilisation of enzymes [3]. The immobilisation of enzymes onto the nanoparticles offers prospects of applications of these nanoparticles as biosensors and biocatalysts [4]. In particular, bio-compatible nanoparticles upon which enzyme has been immobilised are considered to be novel therapeutic and diagnostic agents [5]. Moreover, these nanoparticles make them safe for human application, help to increase the shelf-life of the proteins and to protect them over a longer period of time [6].

Chitosan is well-known as a capping agent due to its significant chemical and biological properties such as hydrophilicity, biocompatibility, biodegradability, and antibacterial properties [7]. Chitosan is safe and non-toxic and therefore widely used in food and bioengineering industries, enzyme immobilisation, and as a carrier for controlled drug delivery [8,9]. The presence of reactive amine and –OH groups in chitosan makes it a good candidate for biomedical and pharmaceutical applications. The passivation layer of chitosan on the surface of nanocrystals makes them bio-compatible so that they can be attached to biomolecules such as DNA [10], RNA, protein [11,12], and peptides [13]. In the present work, nanoparticle encapsulation with chitosan, its size dependent optical properties and immobilisation of trypsin with the encapsulated nanoparticle are discussed, together with the future prospects of such systems. Several studies have been conducted on chitosan capped ZnS:Mn nanoparticles [14–17]. However, no reports are available on the applicability of these bio-compatible nanoparticles in protein immobilisation.

Proteins immobilised on bio-compatible nanoparticles have wide applications in drug targeting and delivery, the isolation of protein inhibitors, peptide mass fingerprinting (PMF) [18], and studying protein–protein interactions. Though proteins are vulnerable to degradation, immobilisation helps to prevent denaturation of proteins. The fluorescence of nanoparticles, attached to the immobilised trypsin also helps to locate the position of protein inhibitors during the isolation process. Therefore, The present work is novel since, there are no previous reports on the application of chitosan capped, fluorescent, ZnS:Mn nanoparticles in protein immobilisation.

Materials and methods

Synthesis of chitosan capped ZnS:Mn nanoparticles

ZnS nanoparticles doped with manganese (Mn) were prepared by a chemical capping co-precipitation method in which zinc acetate [Zn $(CH_3COO)_2{\cdot}2H_2O$] (Merck Specialities Private Limited, Mumbai, >98%), manganese acetate [$Mn(CH_3COO)_2{\cdot}4H_2O$] (Merck Specialities Private Limited, Mumbai, >99.5%) and sodium sulphide $Na_2S{\cdot}9H_2O$ (Merck Specialities Private Limited, Mumbai, 98%) were used as the reactants. These nanoparticles were stabilised by steric hindrances upon using chitosan as a capping agent. The inorganic wet chemical synthesis used to prepare ZnS:Mn nanocrystals was carried out at room temperature in water for all of its inherent advantages of being simple and cost effective. This method is similar to that described by Bhargava and Gallangher [19] with a suitable modification. With Mn doping high luminescence occurs at around 597 nm [20,21]. Chitosan is a bio-compatible capping agent which prohibits the diffusion of ions from the solution and restricts the growth of nanocrystals. In this experiment, 0.1 mol L^{-1} zinc acetate and 0.01 mol L^{-1} manganese acetate were mixed in 50 ml of water along with 0.001% of chitosan to which 0.1 mol L^{-1} sodium sulphide was added dropwise to form chitosan capped ZnS:Mn nanoparticles. The mixture was kept under constant stirring for one and a half hours and then filtered and dried in an oven at 40 °C. A set of samples was prepared by varying the molarity of sodium sulphide from 0.125 to 0.01 mol L^{-1}.

Trypsin assay using BAPNA

Trypsin activity was measured using the synthetic substrate α-N-benzoyl-DL-arginine-p-nitroanilide (BAPNA, Sigma) [22]. For this 25 µl of 0.1 mg/ml trypsin was diluted in 425 µl of 0.01 mol L^{-1} phosphate buffer with pH 7.5. Then 50 µl of 2 mmol L^{-1} freshly prepared BAPNA was added and incubated at

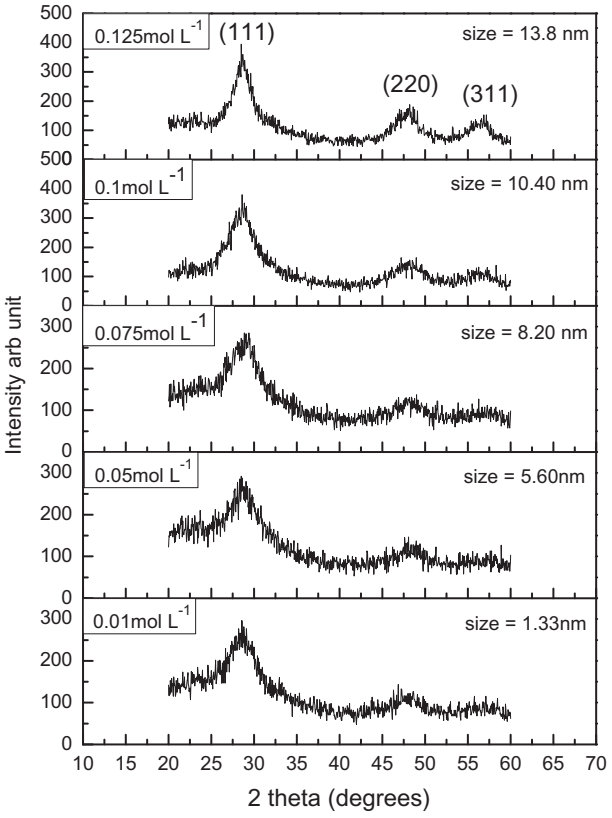

Fig. 1. XRD patterns of chitosan capped ZnS:Mn nanoparticles for various concentrations (0.125–0.01 mol L^{-1}) of sodium sulphide.

Table 1
Spectral characteristics of chitosan capped ZnS:Mn nanocrystals.

Sodium sulphide concentration (mol L^{-1})	Particle size (nm)	Excitonic peak (nm)
0.010	1.33	294
0.050	5.60	308
0.075	8.20	318
0.100	10.4	322
0.125	13.8	327