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Synthesis of Mesoporous Silicon Nanoparticles and Enzyme Immobilization

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ABSTRACT

Mesoporous Silica Nanoparticles (MSNs) provide a non-impassive and biocompatible delivery platform for a broad range of application in therapeutics, pharmaceuticals and diagnosis. In addition, MSNs have proven to be promising support for enzyme immobilization, enabling the enzyme to retain their activity, affording them greater potential for wide range application in bio catalysis and energy conversion. We report here in the synthesis of MSN and its use in enzyme immobilization

KEYWORDS :

Introduction:

The recent research has been demonstrated that bio-molecules immobilized in inorganic matrixes retain their functional characteristics to a large extent, as well as high activities and chemo and regio stereoselectivities. We review on the growing field of amino acids, vitamins, enzymes, and whole cells adsorbed (immobilized) onto ordered mesoporous silica and carbon molecular sieves. There are several reasons for using an enzyme in an immobilized form. In addition to more convenient handling of the enzyme, it provides for its facile separation from the product, thereby minimizing or eliminating protein contamination of the product [1-2]. Immobilization also facilitates the efficient recovery of costly enzymes, in many applications a condition for economic viability, and enables their use in continuous, fixed-bed operation. The adsorption of proteins from solution into solid surfaces has attracted much attention due to its scientific importance and application in many areas. The adsorption (immobilization) of proteins on inorganic materials is crucial because of the potential to improve the stability of enzymes under extreme conditions. The controlled adsorption of proteins is essential in the fields of enzymatic catalysis, biosensors, and disease diagnostics. The discovery of mesoporous silicate molecular sieves opened up new possibilities in many areas of chemistry and material science. These materials possess high specific surface areas, high specific pore volumes, hydrophilic character, water insolubility, chemical and thermal stability, mechanical strength and well-ordered pore structures with uniform miscolours adjustable in diameter from about 1.5 to 30 nm and toxicological safety [3-5]. Besides this achieving retention of the biocatalyst, immobilization is often employed to stabilize the enzyme as translational motion as well as volume-enhancing enfolding of enzymes is restricted. The pore sizes of MSNs are comparable to the diameter of enzymes such as group of proteases, polysaccharide degrading enzymes, lipases, and other industrially important enzymes and for this reason these materials have begun to attract attention as supports for enzyme immobilization. Furthermore, binding to the mesoporous carrier might cause changes in the active site of the enzyme often accompanied by significant loss in catalytic activity.

The mesoscopic regime of 10 to 100 nm represents a largely unfilled gap for three-dimensionally ordered porous materials. Block copolymer templating has been used to access the lower end of this range with porous silicates. Three dimensional materials with pores in the 10-100nm range are of immediate interest for certain fundamental problems, such as the study of diffusion and phase equilibrium in restricted geometries [6]. They also have potential application in the preparation of mesoscopic device. Imprint polymers that retain the shape and size of macromolecular templates have also be reported.

1.2.Various applications of MSN:

a. MSN is biocompatible at the effective dosages and preferentially accumulated in the tumer.

b. Silica is accepted as GRAS by the united states Food and Drug Administration (FDA).

c. Semiconductor nanoparticles have been used for targeted fluorescence imaging of tumour as well as long-term real-time imaging of molecular events in cells [12].

d. MSN nanoparticles with optimum size and appropriate antifouling surface can remain in blood vessels long enough to accumulate at the tumour sites via enhanced permeation and retention effect (EPR). This maximizes their performance in targeted therapy.

2. Objective:

Over the last decades, intensive research and development has focused on the discovery of new bio conjugate by using Mesoporous silica nanoparticles (MSNs) with enzymes. Enzymes, biological catalysts with high selectivity's, are becoming increasingly important in sustainable technology and green chemistry [7-10]. The major limitations in use of free enzymes are their high cost, the stability, the less catalytic activity in the limited range of pH and the degradability by other proteolysis' enzymes. Many research works have already been done in this field by using various enzymes like cellulose, lipase, lysozyme, amylase, catalyse, cytochrome C etc and also different microstructure of MSNs are used for these purpose like MCM-41, SBA-15, FDU-12 etc [11]. Our objective of the research is to synthesis of hierarchical Mesoporous silica matrices suitable for enzyme immobilization.

2.1. Plan of work:

The steps of work are given below orderly:

1. Initially we will select different groups of proteases and polysaccharide degrading enzymes.

2. Synthesis of hierarchical mesoporous silica nanoparticles (MSNs) by sol-gel process or by other suitable methods available in literature.

3. Immobilization experiment with the purified enzymes will be conducted by encapsulation method with our synthesized MSNs with varied morphologies as a pilot investigation.

4. The retention and other enzymatic character will be compared with same amount of un-immobilized enzyme in an identical reaction environment.

5. Comparative studies will also be performed with the commercially available purified enzyme (finally screened) with our prepared mesoporous silica matrix.

3. Materials and methods:

3.1. Reagent:

Tetraethyl orthosilicate (TEOS) (99.99%, Aldrich), ethanol (99.99%, Aldrich), and ammonium hydroxide (28%, Wako) were used without any further purification. Distilled water was used throughout the experiment.

3.2. Synthesis of silica nanoparticles:

The monodisperse uniform-sized silica nanoparticles were prepared by hydrolysis of TEOS in ethanol medium in the presence of ammonium hydroxide. Fig. 1 is a schematic diagram of the synthesis of silica particles and homogeneous samples were prepared by the following procedure. First, ethanol was taken and kept in a fornication bath.

After 10 min, a known volume of TEOS was added while soliciting, and after 20 min, 28% ammonium hydroxide was added as a catalyst to promote the condensation reaction. Sanitation was continued for a further 60 min to get a white turbid suspension. All the above experiments were conducted at room temperature. In the synthetic procedure, the surfactant cetyltrimethylammonium bromide (CTAB) is initially dissolved in basic aqueous solution and the mixture is vigorously stirred at elevated temperature. Tetraethylorthosilicate (TEOS) is added, and the solution is kept stirring at an elevated temperature for 2 h. After the reaction is complete, the as-synthesized product is filtered and washed with abundant water and methanol [13].

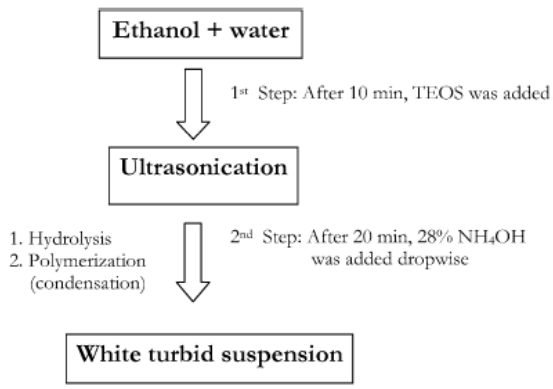

Fig. 1. Preparation of silica particles by sequential addition method.

Fig. 2 illustrates the preparation of different sizes of silica nanoparticles. Fig. 2 represents the effect of TEOS on particle size [14]. Fig. 2 shows that small and uniform-sized particles size 20.5 nm (*SD* < 1.0*)*, were obtained under experimental conditions at ethanol 4M, 0.045MTEOS, and 14Mof NH3. Fig. 2 represents the effect of ethanol concentration on size of the silica nanoparticles.

Silica particles were prepared using a sol–gel process with a sequential addition technique in an ultrasonic bath. Various reaction conditions were adopted in which the concentration of one reagent was fixed and the concentrations of other reagents were changed one by one.

3.3. Characterization:

Primarily the product that obtained is characterized IR spectroscopy. The SEM and TEM of the samples are going on.

4.RESULT AND DISCUSS:

The IR spectroscopy only suggested that the particles are strongly absorbed by the CTAB molecule Fig.3). Results are yet to be received for a meaningful conclusion.

IR SPECTROSCOPY:

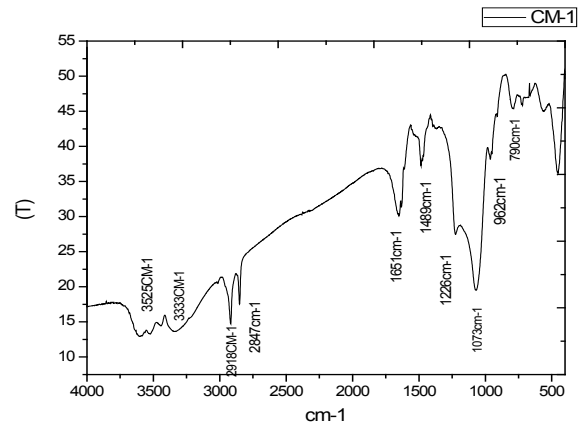

Fig:3:IR Spectra 0f CTAB+MSN

4.1.Observation:

IR: Fourier-transform infrared (FTIR) spectroscopy for surface chemistry of the nanoparticles was recorded in KBr pellets on a Parkin Elmar 1000 instrument.

Surface chemistry of the nanoparticles was studied by FTIR spectroscopy. The results are collected in for SNPs and the characteristic peaks at 1073, 962 and 790 cm^{-1} are attributed to ν (Si–O–Si), ν (Si–OH) and δ (Si–O–Si), respectively. The peak at 3400 cm^{-1} is assigned to surface O–H, ν (O–H), or adsorbed water molecules on SNPs. The peak at 1651 cm^{-1} is due to asymmetric vibrations of H–O–H which is overlapped with vinyl ν(C=C) vibrations. Unfortunately, it seems practically impossible to have dry silica nanoparticles for better resolved ν(C=C) vibrations at this region even after several days of dehydration under reduced pressure possibly due to highly hygroscopic nature of the silica nanoparticles. Two other characteristic peaks located at 2991 cm^{-1} for symmetrical vinyl C–H stretching and at 547 cm^{-1} for out-of-plane deformation vibrations of H atoms on –CH=CH2 provides evidence for surface vinyl functionality. The band at 1411 cm^{-1} is attributed to δ(C–H) which can be attributed to any organic residues, Si–OR or Si–R and it appears in both modified and unmodified silica nanoparticles. The peaks between 2900 and 3100 cm^{-1} are due to ν(C–H). The observed peaks from O–H vibrations in both unmodified and modified silicas confirm the existence of adsorbed water or remaining silanol O–H groups on the particles.

5. Conclusion:

Although quite young, the field of Mesoporous silica doped with biologically interesting molecules has already exhibited its diversity and potential applications in many frontiers of modern materials science including bio catalysis, bio sensing, drug release, and separation of biological molecules. It has been shown that ordered Mesoporous materials are useful for stable entrapment of bio functions and the stabilization of biological interesting molecules under severe conditions. However, the activity of immobilized enzymes is often found to be lower than that of the free enzyme. Work is in progress in our laboratory. We are expecting for a hierarchical MSN for effective immobilization.

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