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Preparation of silane functionalized mesoporous hollow silica nanospheres as drug carriers in photo dynamic therapy

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Abstract
Photodynamic therapy (PDT) is a minimally invasive procedure where the photochemical reaction between light wave, photosensitizer (PS) and ground state oxygen produces toxic singlet oxygen ($^1O_2$. Selective accumulation of PS at the target site is one of the major requirements for effective PDT. Mesoporous silica nanoparticles (MSNPs) have exceptional physiochemical properties for application as nanocarriers in targeted PS delivery systems. In this study mesoporous hollow silica nanospheres (MHSNS) of uniform morphology, high surface area and dimensions about 100 nm were synthesized using the double template method. In order to facilitate covalent bonding with PS the surface of the synthesized MHSNSs were modified with 3-aminopropyl triethoxysilane (APTES) as the silane coupling agent. The functionalization process was optimized by varying different reaction conditions.

Keywords: Mesoporous Hollow Silica Nanospheres, APTES, Photodynamic Therapy, Targeted Drug Delivery

1. Introduction

Photodynamic therapy involves selective accumulation of photosensitizers in target site followed by light irradiation which produces cell damaging singlet oxygen. For effective PDT the photosensitizers should be able to produce high level of singlet oxygen at the targeted tumor cells. A large number of chromophores capable of producing high singlet oxygen yield are available but most of them are hydrophobic and have limited solubility in water. This inability of the PS to accumulate at the target site in sufficient amount reduces the efficiency of PDT. Another important aspect of drug delivery systems is the selective accumulation of drug at the target site to reduce side effects by damage to healthy cells. Accelerated growth of tumors through rapid angiogenesis causes highly permeable blood vessels, leaky cell membrane and poor lymphatic drainage [1]. This enables to selectively accumulate in the tumor cells by enhanced permeability and retention (EPR) effect. The Bio distribution and pharmacokinetics of drugs is largely dependent on its size. The drug circulation time in body fluid can be controlled by adjusting the size of the drug particles. Particles with dimensions smaller than 150nm can easily move through tumor cells. On the other hand particles dimensions smaller than 5.5 nm are easily removed by the excretory system [2]. A longer drug circulation time results in higher drug accumulation.

MSNP are very efficient carriers for drug delivery systems. MSNP are non-toxic, biocompatible and has high oxidation resistance. The tunable mesostructure provides high specific surface area, tunable pore size distribution and its versatile surface chemistry renders easy functionalization. MSNP as a carrier system for the PS molecule can produce an effective targeted delivery system. In recent years MSNP-PS conjugates has been explored to increase the PDT efficiency. Gu *et al.* demonstrated bacteria inactivation by PDT using PS grafted silica nanoparticles [3].

MSNP of different morphologies have been successfully applied in catalysis, separation, drug delivery and therapeutics. Mesoporous silica particles were first reported in 1971 but the development of a series of ordered mesoporous materials in 1992 brought the unique physiochemical properties into limelight. The following table lists the most prominent mesoporous silica particles and their structural properties [4].

Table 1: Most Common mesoporous silica materials

Name	Structure	Pore size(nm)
MCM 41	2-D hexagonal	1.5-10
MCM 48	3-D cubic	1.5-4.6
SBA 1	3-D cubic	1.5-3.0
SBA 3	2-D hexagonal	1.5-3.5
SBA 12	3-D hexagonal	3.0-5.0
SBA 15	2-D hexagonal	4.0-15
SBA 16	3-D cage-like cubic	4.7-12
KIT-1	Disordered	3.4
KIT-6	3-D cubic	4.0-11.5

MSNP are non-toxic, biocompatible and has high oxidation resistance. The tunable mesostructure provides high specific surface area, tunable pore size distribution and its versatile surface chemistry renders easy functionalization. Mesoporous hollow silica nanospheres (MHSNSs) have very high specific surface area with stable pore structure. Different functionalities can be adsorbed or conjugated to both the external surface area and the internal mesopore channels. The hollow structures provide more surface area for drug loading and functionalization compared to the solid MSNPs. This makes the MHSNP a better candidate as drug delivery platform for PDT.

The surface of the prepared MHSNSs is covered with silanol groups which facilitate facile functionalization either by covalent bonding or hydrogen bonding. It has been reported that 90% of the silanol groups are located on the internal pore surface of the MHSNSs. This presents a unique opportunity of selectively functionalizing MSNP with different functional groups by appropriate functionalizing method [5, 6]. 3-aminopropyl triethoxysilane (APTES) is a prominent silane coupling agent used to modify the MSNP surface for enhanced PS bonding. In recent studies minor variation of two prominent functionalization methods; grafting and co condensation have been discussed. In both cases the alkylsilanes bind to the silica surface through Si-O-Si bonding between the silanol grops on the surface and the hydrolyzed silanol groups of the alkylsilanes. The silane contains a hydrolysable siloxy head, hydrophobic propylene bridge and a hydrophilic amino head. The siloxy head hydrolyses and reacts with the silanol groups on the MHSNSs surface. The amine groups are available for further covalent attachment with PS. In this study a targeted PS carrier system for PDT was developed by synthesizing silane modified MHSNSs of dimensions about 100nm and uniform morphology.

2. Materials and Method:

2.1 Synthesis of mesoporous hollow silica nanospheres

In our study MHSNSs were synthesized using a dual template method as described in the literature with minor variations. Pre synthesized CaCO3 nanoparticles with average diameter of 80 nm was used as the form directing particle and cationic surfactant CTAB was used to induce mesoporous structure. The CaCO3 nanocubes were washed with deionized water and dried. The particles were then passed through a 250 wire mesh in order to remove large lumps. 2 g of CaCO3 template was dispersed in 100 ml solution of ethanol and deionized water (Vethanol/Vwater= 1:3).

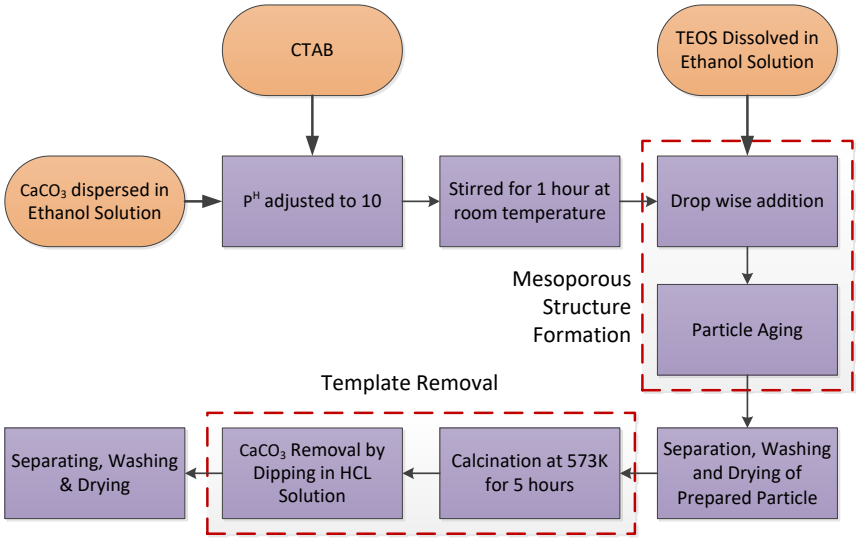

Fig 1: Flow diagram of the synthesis procedure of MHSNSs.

0.1 g CTAB was added to the mixture after adjusting its pH value to 10 by ammonia solution. The mixture was then stirred for 1 hour at room temperature. A mechanical stirrer was used to stir the reactant mixture at a constant 1000rpm. Then 1ml of 3-aminopropyl-Triethoxysilane (TEOS)dissolved in 30 ml ethanol solution was added drop wise to the mixture under constant stirring at room temperature. The TEOS dropping was very carefully monitored to attain very slow and uniform TEOS addition about one drop per 30 seconds.

After the addition of TEOS the mixture was stirred for another 2 hours and then kept at rest for 24 hours. The additional stirring and aging of the mixture facilitates a more stable and high quality mesostructure however a too long aging period can result in agglomerates.

The strength and stability of the mesoporous structure depends on the surfactant to silica ratio. A lower surfactant to silica ratio improves the stability of the mesostructure, increases framework thickness and decreases agglomeration [7]. MHSNSs were synthesized using different surfactant to silica ratio for obtaining a stable mesoporous structure. Surfactant to silica ratio was varied in the range of 0.07(w/w) to 0.27(w/w). Long aging period facilitates silica shell growth hence longer aging period of 48 h was also used to obtain a more stable structure.

The resulting particles were filtered by centrifugation, washed with water and ethanol. The particles were dried overnight at 373K.

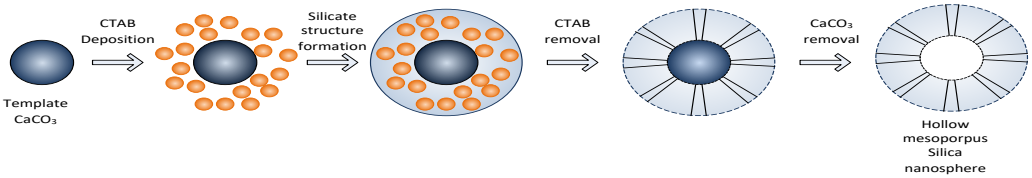

Fig: Error! No text of specified style in document. Diagram illustrating the formation of mesoporous hollow structure by the dual template method.

The dried particles were calcinated for 5 hours at 973K using a heating rate of 1° per minute. The calcination process removed the surfactant template giving rise to a mesoporous silica structure. The particles were then immersed in 0.25 M hydrochloric acid solution and kept at rest for 24 h. The CaCO3 templates dissolved in the acid solution resulting in a hollow core. The mesoporous particles were very carefully added to the acid solution to avoid vigorous dissolution of the CaCO3 template. Excessive high rate of template removal might damage the particle structure. After template removal the particles were then filtered by centrifuge, washed and dried at373K.

2.2 Characterization of the prepared MHSNSs

The morphology of the as prepared MHSNSs was characterized by SEM, powder XRD spectrum and N2 sorption isotherm analysis. The SEM images were used to determine the size and shape of the prepared particles. The XRD spectra and N2 sorption isotherm were analyzed to understand the characteristics of the mesoporous structure.

2.3 Surface modification of the prepared MHSNSs

The as synsethized MHSNSs surface was modified with a silane compound containing - NH2 end groups to prpmote covalent bonding with PS molecules. APTES (3-aminoprropyl triethoxysilane) is one of the most effective organosilanes for developing a amine terminated silica layer [8] .At present a number of well-established methods for amine functionalization with APTES are available [9]. To identify the most suitable functionalization method for our purpose 4 different methods with varied reaction time, temperature, pH and solvent were used to functionalize the prepared MHSNSs.

2. 3.1 Method A

Dry MHSNSs (0.23 g) was dissolved in 40 ml ethanol. 4 ml of 0.8 M ammonia solution in ethanol was added to adjust the pH of the mixture. 0.3ml of 3-aminopropyl triethoxysilane (APTES) was added and the solution was stirred at a rate of 250 rpm at room temperature for 12 h. The particles were then filtered by centrifuge, washed and dried over night at 373K.

2. 3.2 Method B

APTES (0.3 ml) was mixed with 0.23 g of MHSNSs dissolved in ethanol. The mixture was stirred at a rate of 250 rpm for 24 hours at room temperature. After filtration by centrifugation the particles were washed and dried at 373K overnight.

2. 3.3 Method C

Dry MHSNSs (0.4 g) was dissolved in a solution of ethanol in water. 0.6 ml APTES in ethanol was added to the mixture. The mixture was then stirred at 348K for 5 hours. The resulting particles were filtered, washed and dried overnight.

2. 3.4 Method D

Dry MHSNSs (0.25 g) was dispersed in 100 ml toluene in sonic mixer for 30 minutes. Then, 0.7 ml of APTES was added and the solution was refluxed in nitrogen atmosphere at 398 K for 24 hours. The particles were then filtered, washed and dried overnight.

Fourier transform infrared (FTIR) spectroscopic analysis was used to detect the more effective amine functionalization method among the four methods investigated. In order to further improve functionalization the MHSNSs were functionalized by the toluene based method (method D) using varying amounts of APTES in the reaction mixture. The APTES content was varied from 0.1 ml to 0.7 ml in order to find the optimal amount of APTES in the reactant mixture.

3. Results and discussions:

Scanning Electron microscopy images of the prepared MHSNSs were used to study the morphology and structure of the particles. The SEM micrographs in Fig. 2-3 shows hollow spheres of around 100 nm diameter were formed. The particles were comprised of a mesoporous silica shell around a hollow core. Broken shells in some of the formed spheres indicate that the silica shell is not very strong and might have ruptured due to the rapid rate of the template (CaCO3) removal in acid solution. The MHSNSs formed with low surfactant to silica ratio (Fig. 2-3-a) produced more stable and thicker mesoporous silica wall. Whereas higher surfactant ratio (Fig. 2-3-c and d) produced thin mesostructure shells resulting in more particles with broke shells. We also tried to improve the MHSNSs morphology by controlling the stirring speed and aging period. Very high stirring speed and long aging period resulted in high agglomeration with thin shell thickness (Fig.2-3-e).

One of the setbacks of the prepared MHSNSs was that it was not monodisperse, the particles formed small coagulates (Fig 2-3a, b, c, d). To overcome this drawback many variations of the preparation method such as the reactant ratios, stirring speed, aging time, template removal method has been studied but monodispersity could not be achieved. It was reported by Kim *et al* that using a dispersing agent such as tri block copolymers, monodispersity of silica nanospheres could be attained [3].

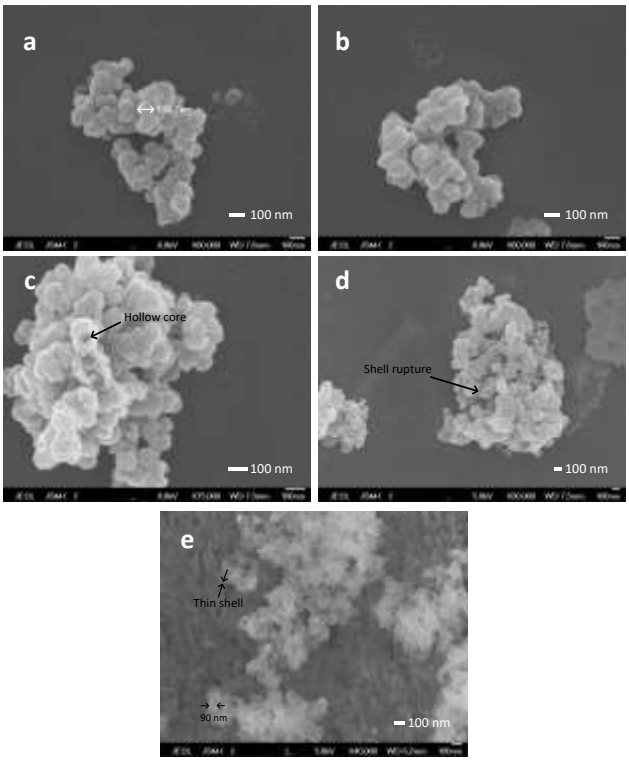

Fig 2: SEM images of prepared MHSNSs. Particles formed using low surfactant ratio (a and b), high surfactant ratio(c and d) and particles formed at high stirring speed with long aging time(e)

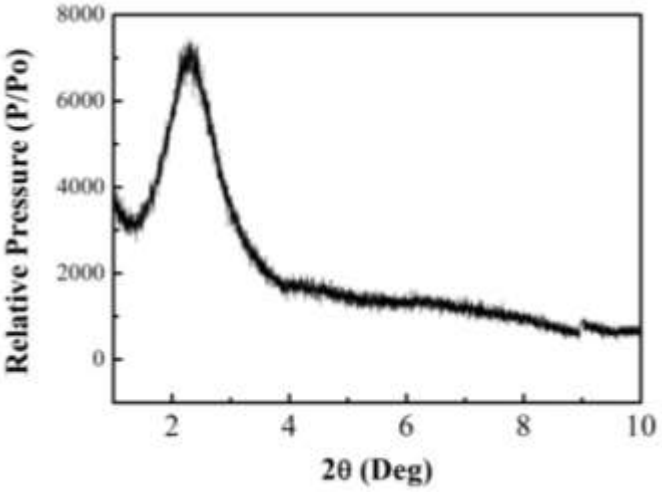

Fig 3: Powder XRD pattern of the synthesized MHSNSs.

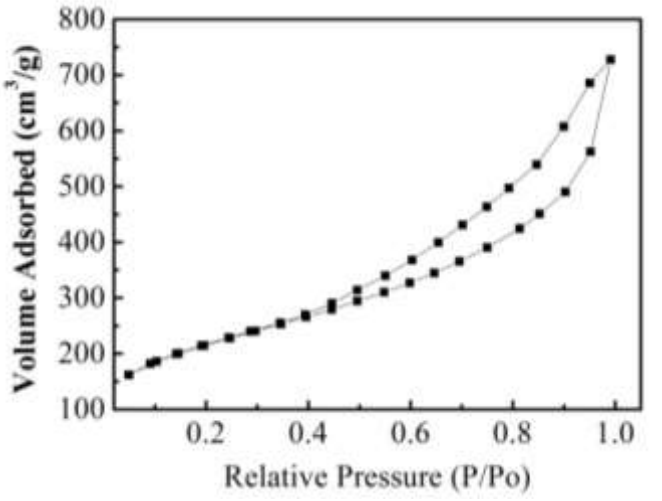

Fig. 4: N_2 adsorption-desorption isotherms of the prepared MHSNSs.

Powder XRD patterns of the as synthesized MHSNSs shows that a broad peak exists around 2θ= 2.5°. This indicates mesoporous silica structure lacking long range order in the mesostructure [10].

The Nitrogen adsorption analysis gives a better insight to the structural properties of the as prepared nanoparticles. The nitrogen adsorption desorption isotherms resulted a typical type IV isotherm according to the IUPAC classification. A hysteresis loop was observed between the relative pressures of 0.3 to 1. This is a characteristic isotherm for mesoporous structure [11]. The BJH (Barret-Joyner-Halenda) method was used to determine the pore size distribution [12]. The PSD curve shows that the prepared mesoporous silica particles have a broad pore size distribution. Although there are a few larger pores around 8-10 nm most of the pores have diameter around 3.7 nm. The pore volume of the MHSNSs particles as calculated by the BJH method is 1.105cc/gm. Specific surface area was calculated using the BET method from the nitrogen sorption isotherms. The synthesized MHSNSs have a surface area of 789.63 m^2/g.

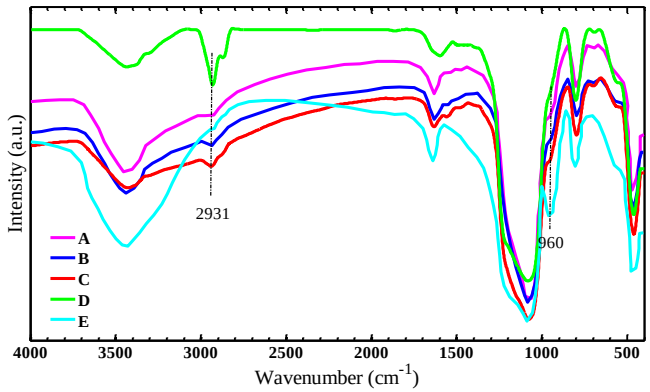

Fig 6: FTIR spectra of the particles functionalized by method A (A), method B(B),method C(C), method D(D) and the MHSNSs.

Fig. 3-6 shows the FTIR spectra of the as prepared MHSNSs and the amine functionalized particles with the 4 different methods. The as prepared MHSNSs show a large peak **at** 960 cm^{-1} corresponding to silanol groups (Si-OH) on the surface. After functionalization this peak decreases suggesting covalent bond formation with the alkyl silanes. All the 4 methods exhibited similar reduction of Si-OH groups on the MHSNSs. The covalent attachment of APTES is indicated by the presence of peaks corresponding to aminopropyl groups [13] .The stretching vibrations of the C-H bond in the propyl group exhibit peak in the 2800 -3000 cm^{-1} range. The FTIR spectra shows distinct peaks at 2936 cm^{-1} corresponding to the presence of aminopropyl groups in all the amine functionalized particles. Method A, B and C produced similar results but method D using toluene as solvent produced considerably sharper peak. The methods A, B and C were performed by using different reaction conditions such as pH, heating, reaction time while using the same reactants and solvents. But in method D the reactants were dissolved in toluene and refluxed in N_2 environment. This provided significantly improved functionalization as indicated by the sharp peak at 2936 $cm^{-1.}$

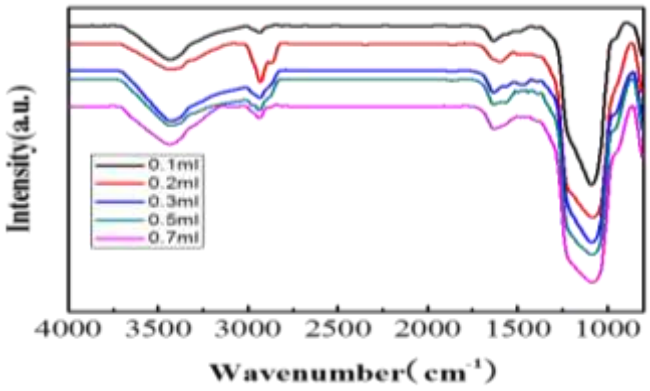

Fig 7: FTIR spectra of the particles formed by functionalization with varying amounts of APTES soln.

FTIR spectra (Fig 3-7) of the resulting MHSNSs-NH_2 particles show a sharp peak at 2930 cm^{-1} corresponding to amminopropyl group and decreased peak at 960 cm^{-1} indicating a reduction in available silanol groups on the

MHSNSs surface. The MHSNSs functionalized by 0.2 ml APTES in the reaction mixture generates the sharpest peak at 2930 cm^{-1} among the functionalized particles.

4. Conclusion

MHSNSs particles prepared using the dual template method with CaCO3 and CTAB as templates were characterized by SEM, XRD and N_2 sorption isotherms. Spherical particles with diameter around 100 nm and a hollow core were synthesized. The size of the prepared MHSNSs is appropriate for therapeutic applications as nanoparticles in the size range of 100-200 nm have maximum selective accumulation and cellular uptake.

The prepared MHSNSs have a stable meso structure with very high specific surface area and pore volume. The specific surface area calculated by BET method was 789.63 m^2/g and the pore volume calculated by the BJH method was 1.105 cc/gm. The powder XRD patterns and N2 sorption isotherms also indicated the formation of mesoporous structure lacking long range order.

The MHSNSs were functionalized by the grafting method. APTES was used to functionalize the MHSNSs surface with $–NH_2$ groups. Different methods for functionalization varying in reaction temperature, time, pH and solvent were investigated. Analysis of the FTIR spectra indicated that the method using toluene as solvent under reflux in N_2 atmosphere produced the best results. The prepared MHSNS-NH2 has great promise as a PDT agent. It has the appropriate, size, morphology and physiochemical properties required for efficient drug delivery in PDT. But we were unable to produce monodisperse particles. The Use of dispersing agents to acquire monodisperse particles may be further investigated.

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