

Green Synthesis of Activated Bovine Serum Albumin Fluorescent-Nanosphere by Using Mustard Oil as Herbal Nano-Alternative

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Abstract

These days, preparation and characterization of various nanostructures are under many scientific considerations to get controllable geometry and their smallest size at nanoscale. Protein nanoparticles have been proved a effective and improved drug delivery vehicle for site specific sustained and targeted delivery. Many chemical and green practices of nanostructures such as nanocrystals, nanosphere, nanodiscs, nanocubes, nanowires, nanoballs and nanorods are going to be fabricated with many biocompatible and safe metals ions (gold, silver, iron and copper) and biomaterials (carbon nanotubes, chitosan, albumin and lipopolysaccharides and polysaccharides). These designed nanostructures along with fabricated metal ions/polymers/albumin were subjected further for many considerable improvements especially for the oxidative etching effect in nanostructures. It is reported that it may play very implicative role in fabrication of nanostructures geometry designing and their characterization for their size and attained shapes. Previously, bovine serum albumin was used as a cost effective matrix for preparing the various nanoparticles due to having couple of exploitable characteristics e.g. biocompatibility, non-antigenicity, easily biodegraded and non-toxicity. This designed study can provide a potential green method to prepare nanosphere that find to be assist in controlling desired size and shape of bovine serum albumin nanospheres at nanoscale by using mustard oil which is a naturally occurring and affordable antibactericidal emulsifier. Characterization of prepared glutaraldehyde activated bovine serum albumin luminescent nanospheres was done with Scanning Electron Microscopy (SEM) to observe their exhibited size and shape. So, this proposed method can be proved a very much cost effective green technology to prepare non-toxic and biocompatible activated bovine serum albumin luminescent-nanosphere to explore their therapeutic approaches in regenerative medicine and nanomedicine. And, It can be used as nonviral gene or drug nanosacs/nanocages to carry out the site specific delivery.

Keywords: Bovine serum albumin, Mustard oil, Nanospheres, Nanoballs, Nanocubes, Nanodiscs, Nanocystals, Scanning Electron Microscopy.

Introduction

Protein nanoparticles have been proved a effective and improved drug delivery vehicle for site specific sustained and targeted delivery by ligand attachment. Non steroidal anti-inflammatory drug (NSAID), Piroxicam (PRX) is found to be assembled with bovine serum albumin nanospheres which was prepared by using a very simple, rapid desolvation method. These formulated BSA-PRX nano particles was exhibited a uniform spherical shape with an average size of 388.7nm and their in-vitro release of loaded drug from BSA conjugate was of about 50% delivery rate of the drug during first 3h and 85% after 18h¹. In other study, calcium loaded bovine serum albumin (BSA) nanoparticles were also prepared using a modified desolvation method and reported approximate size range was found in-between 100-800 nm. The size and the surface-area-to-volume-ratio of the calcium loaded bovine serum albumin nanoparticles were controlled by adjusting bovine serum albumin concentration, pH and NaCl content and observed a useful parameter to estimate the effectiveness of delivery done by prepared nanoparticles². Green synthesis of carbon

nanocubes/nanoparticles were also done by using pyrolyzing rice powder at 600°C under nitrogen atmosphere and characterized by XRD pattern, SEM and TEM, Solid state electronic spectrum. Solid state electronic spectrum was also carried out to that showed several bands in the ultraviolet and visible region and excitation at 336 and 474 nm which generates photoluminescence respectively in the ultraviolet and visible region³. Poorly water-soluble drugs such as nifedipine was also fabricated as nifedipine nanoparticles with high pressure homogenization. Crystalline state evaluation before and following particle size reduction was also performed followed with differential scanning calorimetry (DSC) and powder X-ray diffraction (PXRD) to assigned various participating eventual transformation to amorphous state during the their synthesis⁴. Moreover, due to defined nanostructure of protein and albumin based nanoparticles are offered various possibilities for safe and cost-effective surface approach including covalent loading/binding of drugs and tagging photoluminescent ligands to be considered as effective site specific nonviral tagged loading vehicle used in cancer, tumor, neurodegenerative and spinomuscular diseases gene/drug therapy. Cationic bovine

serum albumin based self-assembled hybrid-nanoparticles were also prepared as siRNA delivery vector and had been used for treating lung metastatic cancer as low cost and nontoxic nonviral gene delivery vehicle⁵. The geometry and size of nanostructures are found to be affected by changing the concentration of catalysts e.g. metals ions, polymers and albumin that directly affects the localized surface plasmon resonance and surface-enhanced Raman scattering. The process of development of synthetic routes used for the formulation of multimetallic nonmetallic/polymeric nanostructures such as nanocrystals, nanospheres, nanoballs, nanocubes, nanodiscs, nanobricks, nanowires and nanorods are found to be big challenge to optimize them at nanoscale with desired geometry^{6,7}. Previously, various polymeric nanoparticles/nanocrystals were also designed to prepare for loading of desired enzymes or drugs into biocompatible and cost effective matrix e.g. chitosan, gelatin, sodium alginate, ficoll, sepharose and albumin which are proposed to be used for nanotherapeutic approaches as suitable targeted nonviral gene/drug delivery carriers⁷⁻⁸. Various drug delivery nanovehicles had been proposed to be used as potential site specific delivery of loaded ingredients. It was found to be having good biodegradability with high loading capacity for prolonged circulation at specific target sites in host cell⁹⁻¹¹. Carbon nanoballs or nanospheres were also synthesized by using arc discharge technique with acetylene with coke powder as carbon source. Their characterization *in situ* was done by Field emission scanning electron microscopy (FE-SEM), field emission scanning and transmission electron microscope (STEM) equipped with energy dispersive X-ray (EDX), optical emission spectroscopy (OES) and Raman spectroscopy and X-ray diffraction (XRD). The STEM results showed that the observed diameter of carbon nanoballs was ranging from 50–100 nm¹². Synthesis of bioactive stable enzyme or drug loaded nano-bovine serum albumin and Egg nanoparticles was also proposed that can be successfully used as eco-friendly, low-cost and non-toxic drug delivery trigger to deliver the loaded biological and chemical materials at targeted sites¹³⁻²⁰. Hence, this proposed study was designed to develop the cost effective and non-toxic slightly modified green method of preparing bovine serum albumin luminescent-nanospheres by using mustard oil as low-cost, easily available, non-allergic, antioxidative and antibactericidal biocatalyst. Characterization of synthesized mustard oil driven bovine serum albumin luminescent-nanospheres was done with Scanning electron Microscopy (SEM) for the observation of their exhibited shape and size. These glutaraldehyde activated bovine serum albumin luminescent-nanospheres were prepared by proposed eco-friendly and low-cost green method that can be used as non-toxic drug/ gene delivery nonviral nanocages or nanoframes when any desired drugs/biological/formulated chemical materials/gene might be bind into them. These glutaraldehyde activated bovine serum albumin luminescent-nanospheres can be used as safe and potent targeted drug/gene delivery nonviral carriers that can be used in desired clinical therapeutic practices in field of regenerative medicine and nanomedicine.

Materials and Methods

Green synthesis of mustard oil driven activated bovine serum albumin luminescent-nanospheres: Mustard oil bath was prepared with slight modification of previous methods used by Rani, K and Chauhan, C., 2015¹⁸; Rani, K and Chauhan, C.¹⁹; Rani, K²⁰. For preparing mustard oil bath, 50 ml of olive oil was mixed with 25% glutaraldehyde. Then, 8-10 ml of bovine serum albumin solution was taken in 10 gauge syringe and added into the prepared bath. Then, it was incubated overnight with constant stirring. Next day, it was further subjected to sonication for 25 minutes with 2.6 ml of n-butanol with slight modification to minimized the size and structure of nanospheres¹⁸⁻²⁰.

Characterization of mustard oil driven activated bovine serum albumin luminescent nanoballs by Scanning Electron Microscopy (SEM): The prepared activated bovine serum albumin luminescent-nanospheres were subjected to Scanning Electron Microscopy (SEM) for the interpretation of their particle size and exhibited shape^{3,11,18-20}.

Results and Discussion

Characterization of mustard oil driven activated bovine serum albumin luminescent-nanospheres by Scanning Electron Microscopy (SEM): Characterization of mustard oil driven activated bovine serum albumin luminescent-nanospheres was done with Scanning Electron Microscopy (SEM) for assigning their exhibited size and geometry (Figure-1). Scanning Electron Microscopy (SEM) result of prepared activated bovine serum albumin luminescent-nanospheres was found to be observed their exhibited size in the range of up to 78.8 nm and exhibited perfect uniformed round shape of prepared nanospheres (Figure-1). Their SEM observations for their sizes are very much similar with previous SEM studies^{3,11,17-20}. The observed colour of the prepared nanospheres was found to be off white having visible unique fluorescent property (Figure-1).

This fluorescence was observed due to formation of diene type of adduct that leads to exposure of tryptophan residue (hydrocarbon that exhibited two carbon double bonds). This fluorescent/luminescent diene type of adduct is formed after the covalent coupling done by using glutaraldehyde (cross linking agent) to activate these bovine serum albumin nanospheres which can be further subjected to allow binding of other desired compatible biological or chemical component. This formed luminescent adduct is reported to be consisting tryptophan, tyrosine and phenylalanine among which tryptophan residue acts as a free acid and has much stronger fluorescence as compared to tyrosine and phenylalanine. This SEM observation was very similar to previous report that confirmed these kind of fluorescent bovine serum albumin nanoparticles¹⁶.

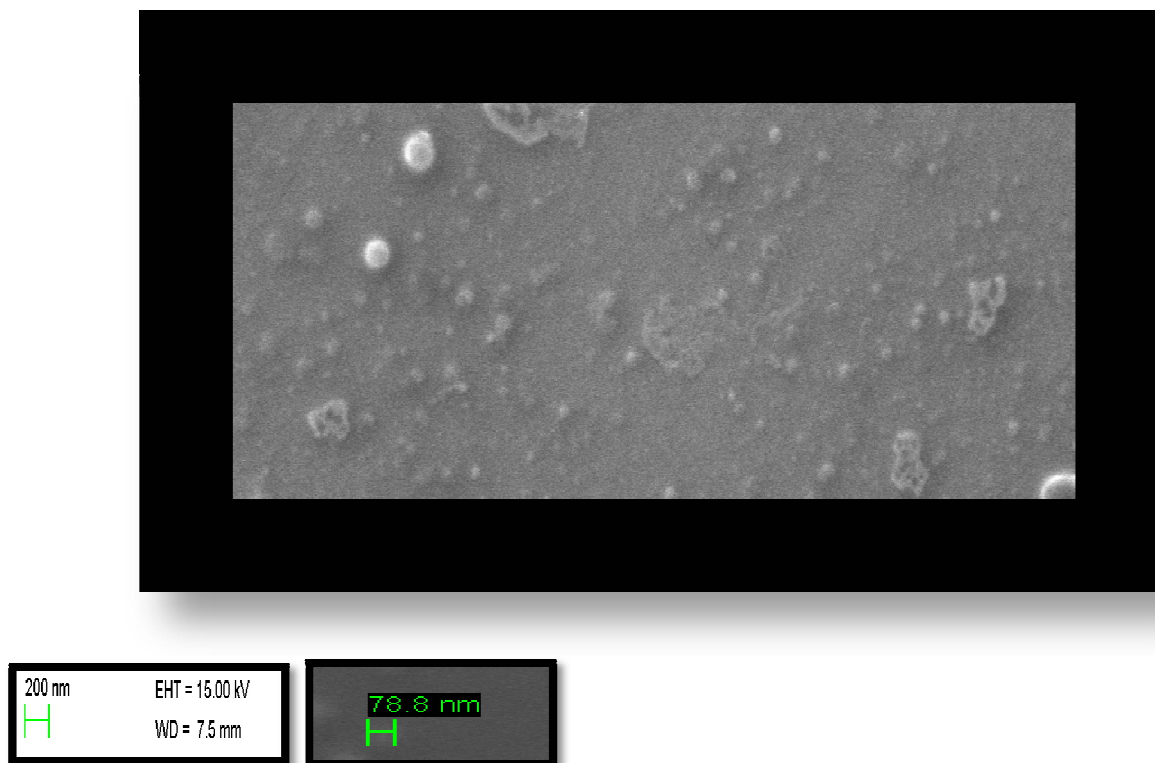


Figure-1
SEM result of mustard oil driven activated bovine serum albumin luminescent-nanospheres

Hence, this observed luminescent activated bovine serum albumin nanospheres may be loaded with desired drug or spliced gene that can be very helpful to trace the drug or gene trafficking in the host cell or genome easily via recording of its photoluminescent signals with fingerprinting and trace explosive detection technologies. No much requirement of any photoluminescent metals, charged polymers and ligands are needful to be attached with prepared drug and gene bound nanoparticles for mapping site specific targeted drug delivery in host cell or genome.

Conclusion

Hence from this proposed work, it was concluded that green synthesis of activated bovine serum albumin luminescent-nanospheres can be proved easy, cost-effective and safe as well as purely herbal alternative over other costly and tedious chemical methodologies. Olive oil was used to prepare these activated bovine serum albumin nanospheres which is antibactericidal, safe and natural biocatalyst which make it more herbal alternative and no side effects in the host cell can be possible if these nanospheres are designed to be administrated any loaded drug in host cell. As well as, this observed luminescent property of these activated bovine serum albumin nanospheres can be proved a safe and herbal green nanotherapeutic approach if loaded any drug or spliced gene that can be easily traced the bound drug or gene trafficking in the host cell or genome via recording of its photoluminescent

signals with fingerprinting and trace explosive detection technologies. In previous studies, photoluminescent metals, charged polymers and ligands are used to tag with drug or gene loaded nanoparticles but sometimes, they might cause toxicity in host cell because of their expected slow degradation. But this proposed study, these ligands/polymers/metals are required to be attached with prepared drug and gene bound nanoparticles for mapping site specific targeted drug delivery in host cell or genome. And, this proposed green method of synthesizing the activated bovine serum albumin luminescent-nanospheres may be proved new green and herbal revolution in field of nanomedicine and molecular medicine.

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