Fourier Transform Infrared Spectroscopy (FTIR) Spectral Analysis of BSA Nanoparticles (BSA NPs) and Egg Albumin Nanoparticles (EA NPs)

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Abstract

Advance protein based nanobiotechnology are now, known for its advanced approach to synthesize various nanomaterials by using various newly developed chemical and green technologies to carry out their applications in various fields of life-sciences. Now these days, Bovine serum albumin nanoparticles (BSA NPs) are very popular as non-toxic and nonviral vehicle systems for drug delivery used in number of therapeutic strategies to be considered in combating cancers, tumors, hormone associated and neurodegenerative disease. The albumin nanoparticles e.g. bovine serum albumin nanoparticles (BSA NPs) and egg albumin nanoparticles (EA NPs) are easily prepared by number of established emulsified and desolvation process to get desired particle size at nanosacle e.g. range between 100 to 300 nm with minimal size distribution. The size controlled BSA and egg albumin nanoparticles can be used as the standard cost effective, non-toxic, non-allergic and non-viral biocompatible nanodevice and nanovehicle carrier for loading of desired drug/ biological active ingredients/ hormones molecules/ chimeric-DNA with efficient target delivery system. As well as, fabrication of BSA NPs and EA NPs by using emulsified and desolvation method are done via glutaraldehyde coupling for loading of Cicer arietinum amylase that found to be better eco-friendly and cost-effective choice as compared to synthesis of other metal based nanoparticles. Sometimes, the metalonanoparticles or nanomaterials are found to be very costly and toxic because of their slow natural degradation in host and their chemically driven synthesis. Hence, the synthesis of bio-compatible BSA NPs and EA NPs might be used for their further consideration for their safe and non-toxic therapeutic applications in fields of biosensor technology, biomedical approaches and pharmaceutical industry for the treatment of various diseases. Hence, this designed Fourier Transform Infrared Spectroscopy (FTIR) analysis was used to determine the purity of the prepared bovine serum albumin nanoparticles (BSA NPs) and egg albumin nanoparticles (EA NPs) and their active functional groups and chemical interactions for loading desired biological components or drugs that can might be further considered to increase their safe and cost effective therapeutic viability.

Keywords: Bovine serum albumin nanoparticles, Egg albumin nanoparticles, BSA NPs, EA NPs, Desolvation method, Emulsification method, Cicer arietinum amylase, Drug delivery, FTIR.

Introduction

Fine particles are considered to be known which are sized in-between 100 and 2,500 nanometers and ultrafine particles are purely called nanoparticles which were found to be in the range of 1-100 nanometers in size. These days, protein based nanotechnology are very much considered to the research and technological developments at atomic, molecular and macromolecular scales to prepare synthetic polymers together with the absorbability and low toxicity of the degradation end products. Silver-poly(methylmethacrylate) (Ag/P MMA) nanocomposites were prepared via in-situ polymerization technique using N,N'-dimethylformamide (DMF) and characterized by Fourier transform infrared spectroscopy (FTIR) to know the details of active functional groups present in the synthesized PMMA. FTIR spectra of silver nanoparticles was also found to be observed to have exhibited prominent peaks at 2.927, 1.631, and 1.383 cm⁻¹ which showed sharp and strong absorption band at 1.631 cm⁻¹ assigned to the stretching vibration of (NH) C=O group; developed band 1.383 for C–C and C–N stretching; observed sharp peak at 2.927 cm⁻¹ that assigned to C–H and C–H (methoxy compounds) stretching vibration. FTIR spectra was also used for characterization of ZnO nanoparticles. Infrared studies were carried out in order to ascertain the purity and nature of the metal nanoparticles that had observed peak at 3452.30 and 1119.15 cm⁻¹ which may be present due to O-H stretching and deformation. Other observed peaks at 1634.00 and 620.93 cm⁻¹ were also reported to correspond the Zn-O stretching and deformation vibration, respectively.

And, FTIR spectral analysis of the iron oxide nanoparticles and microgel magnetic particles were also reported to characteristic the absorption band of Fe–O bond of bulk Fe₃O₄ at 570 and 375 cm⁻¹ to reduced the size of prepared nanoparticles to nanoscale dimensions. The molecular characteristics of the resulting BSA NPs were also characterized by FTIR to have sharp FTIR peaks at 3385 cm⁻¹, 3113 cm⁻¹, 1707 cm⁻¹, 1533 cm⁻¹ and 1242
cm-1 that are assigned to the stretching vibration of OH, amide A (mainly NH stretching vibration), amide I (mainly C=O stretching vibrations), amide II (the coupling out phase of bending vibration of N-H and stretching vibration of C-N bonds) and amide III (is in the phase combination of N-H in plane bending and C-N stretching). Reversely, various techniques e.g. desolvation, emulsification, thermal gelation, nanospary drying, nab-technology and self-assembly techniques were used for the preparation of albumin nanoparticles which do not require high temperature and therefore may be useful when heat-sensitive bioactive compounds are designed to be immobilized into fabricated albumin nanoparticles.

Hence, protein nanoparticles are observed to be an attractive biomolecule which have their unique characteristic including biodegradability, non-toxicity, metabolized in vivo to produce innocuous degradation products, water solubility, simplicity in purification and nonimmunogenic nature that allow their ease of controlled and targeted delivery as an ideal nanopreparation. And, desolvation methods are found to be reported more advantageous over emulsification method to prepare albumin nanoparticles due to avoidance of organic solvents to remove the used oily residues and surfactants which are required for the emulsion stabilization. Desolvation technique was found to be more cost effective to prepare more small sized, thermo-stabilized, storage-stabilized nanoparticles with their reported significant alkaline protease mediated controlled biodegradation and increased shelf life period over upto 12-14 months. Previously, the enzyme immobilization was also reported into those prepared BSA NPs and EA NPs nanoparticles which were more cost effective, eco-friendly biocompatible, non-toxic, non-allergic and non-corrosive enzyme loading carrier by using desolvation method and emulsification method to make them more industrially viable.

Hence, this concrete Fourier Transform Infrared Spectroscopy (FTIR) spectral analysis was used for the interpretation of prepared purity of the prepared bovine serum albumin nanoparticles (BSA NPs) and egg albumin nanoparticles (EA NPs) by desolvation method; bovine serum nanoparticles (BSA NPs) and egg albumin nanoparticles (EA NPs) by emulsification method. As well as, their active functional groups and molecular interactions were also assigned to get to know the altered chemical interactions in prepared BSA NPs and EA NPs before and after the glutaraldehyde activation that were further used for desired Cicer arietinum amylase immobilization to be used for their controlled alkaline protease mediated bioproteolysis and effective washing study. So, FTIR analysis was used to carried for the functional chemical interactions of prepared BSA NPs and EA NPs which can might be exploited for their further inevitable use to load the desired drug/ biological active ingredients/ hormones molecules/ chimeric-DNA with efficient target delivery system as cost effective, non-toxic, non-allergic and non-viral biocompatible nanodevice and nanovehicle carriers. That may become a landmark in non-toxic therapeutic applications especially in fields of biosensor technology, biomedical approaches and pharmaceutical industry for the treatment of various diseases (cancer, tumors, hormone-associated and neurodegenerative disorders) as green clinical therapeutic technology.

**Materials and Methods**

**Preparation of BSA NPs and Cicer arietinum amylase loaded BSA nanoparticle (Amylase-BSA NPs) by Desolvation method:** BSA NPs and Amylase-BSA NPs were prepared by desolvation method by using n-butanol given 2012 and Rani K & Chauhan C, 2015.

**Preparation of BSA NPs and Cicer arietinum amylase loaded BSA nanoparticle (Amylase-BSA NPs) by Emulsification method:** BSA NPs and Amylase-BSA NPs were prepared by Emulsification method by using coconut oil as natural occurring emulsifier given Rani K & Chauhan C, 2015.

**Preparation of EA NPs and Cicer arietinum amylase loaded Egg Albumin nanoparticle (Amylase-BSA NPs) by Desolvation method:** EA NPs and Amylase-EA NPs were prepared by desolvation method by using toluene given by Rani K, 2015.

**Preparation of EA NPs and Cicer arietinum amylase loaded Egg Albumin nanoparticle (Amylase-BSA NPs) by Emulsification method:** EA NPs and Amylase-EA NPs were prepared by emulsification method by using using coconut oil as natural occurring emulsifier with toluene and n-butanol, given by 2012 and Rani K, 2015.

**Alkaline Protease Mediated Bioproteolysis of Prepared Amylase-BSA NPs and Amylase-EA NPs:** Amylase loaded BSA NPs and EA NPs were also subjected to proteolysis with standardized 35 U of alkaline protease to confirmed loading of desired amylase into prepared active BSA NPs and EA NPs.

**Characterization:** Fourier Transform Infrared Spectroscopy (FTIR) analysis of prepared BSA NPs and EA NPs: The prepared BSA NPs and EA NPs; Amylase-BSA NPs and Amylase-EA NPs were subjected for characterization to Fourier Transform Infrared Spectroscopy (FTIR) analysis to determine the purity of fabricated BSA NPs and EA NPs before and after the immobilization done by glutaraldehyde coupling. Designed FTIR spectroscopy was carried out to be scanned at the range of 500-4000cm⁻¹.

**Results and Discussion**

Previously reported data of DLS and SEM for confirmed BSA NPs and EA NPs by Rani K & Chauhan C, 2015; Rani, K, 2015: Dynamic Light scattering (DLS) and Scanning Electron Microscopy (SEM) of prepared BSA NPs and Amylase-BSA NPs by desolvation method and emulsification method were already reported by Rani K & Chauhan C, 2015, to confirm the size of nanoparticles in the approximate range of
2nm to 11nm and 56nm to 107.4nm respectively\textsuperscript{11,12}. Dynamic Light scattering (DLS) and Scanning Electron Microscopy (SEM) results of prepared EA NPs and Amylase-EA NPs by desolvation method and emulsification method were observed by Rani K, 2015, to confirm the size of nanoparticles in the approximate range of 5nm to upto 100nm and 101.2nm to 209nm respectively\textsuperscript{13,14}. The observed size of BSA NPs and Amylase-NPs were found to be less than of EA NPs and Amylase-EA NPs\textsuperscript{11-14}. Those observation were also comparable similar to other previous reports\textsuperscript{9,16-18}.

**Fourier Transform Infrared Spectroscopy (FTIR) analysis of prepared BSA NPs and Amylase-BSA NPs & EA NPs and Amylase-EA NPs:** FTIR spectroscopy was used to determine the purity of fabricated BSA NPs and EA NPs before and after the immobilization of desired enzyme done by glutaraldehyde coupling\textsuperscript{11-15}. It was scanned at the range, 500-4000 cm\textsuperscript{-1}\textsuperscript{9,16}.

**Fourier Transform Infrared Spectroscopy (FTIR) analysis of prepared BSA NPs and Amylase-BSA NPs:** The FTIR peaks of prepared BSA NPs by using desolvation method\textsuperscript{11} before immobilization of amylase were observed at 3381 cm\textsuperscript{-1} that assigned to the stretching vibration of OH [amide A, mainly NH stretching vibration]; at 2958 cm\textsuperscript{-1}, at 2932 cm\textsuperscript{-1} and at 2873 cm\textsuperscript{-1} that approximate assigned C–H and C–H (methoxy compounds) stretching vibration; at 1642 cm\textsuperscript{-1} and at 1292 cm\textsuperscript{-1} that assigned to having approximate vibration of (NH) C=O group and for C–C and C–N stretching respectively (Figure-1).

The FTIR peaks of BSA NPs after immobilization of amylase were observed at 3388 cm\textsuperscript{-1} that assigned to the stretching vibration of OH [amide A, mainly NH stretching vibration]; at 1649 cm\textsuperscript{-1} that assigned approximate the stretching vibration of (NH) C=O group (Figure-2). Observed FTIR peaks at 2958 cm\textsuperscript{-1}, at 2932 cm\textsuperscript{-1} and at 2873 cm\textsuperscript{-1} were only observed in BSA NPs before immobilization only (Figure-1) which confirmed that immobilization of enzyme was done at these assigned activated functional groups (Figure-2). These observations were found to be comparably similar with previous FTIR analysis of nanoparticles\textsuperscript{6,9,16-19}.

The FTIR peaks of BSA NPs by using emulsification method\textsuperscript{12} before immobilization of desired enzyme were observed at 3478 cm\textsuperscript{-1} that assigned to approximate stretching vibration of OH [amide A, mainly NH stretching vibration]; at 2923 cm\textsuperscript{-1} and at 2854 cm\textsuperscript{-1} that assigned approximate C–H and C–H (methoxy compounds) stretching vibration; at 1697 cm\textsuperscript{-1} that assigned to having approximate vibration of (NH) C=O group and for C–C and C–N stretching respectively (Figure-3). The FTIR peaks of BSA NPs after immobilization of amylase were observed at 3566 cm\textsuperscript{-1} that assigned to have approximate possible stretching vibration of OH [amide A, mainly NH stretching vibration]; at 1647 cm\textsuperscript{-1} that assigned approximate the stretching vibration of (NH) C=O group; at 1369 cm\textsuperscript{-1} tha assigned to have possible C–C and C–N stretching (Figure-4). Observed FTIR peaks at 2958 cm\textsuperscript{-1} and at 2854 cm\textsuperscript{-1} were only observed in BSA NPs before immobilization only (Figure-3) which confirmed that immobilization of enzyme was done at these assigned activated functional groups (Figure-4). These reported were found to be comparably similar with previous FTIR interpretation of nanoparticles\textsuperscript{6,9,16,20}.

FTIR spectra of prepared activated BSA NPs by desolvation method before the immobilization of *Cicer arietinum* amylase

\textbf{Figure-1}
Figure-2
FTIR spectra of prepared activated BSA NPs by desolvation method after the immobilization having loaded *Cicer arietinum* amylase (immobilization done by glutaraldehyde coupling)

Figure-3
FTIR spectra of prepared activated BSA NPs by emulsification method before the immobilization of *Cicer arietinum* amylase
Fourier Transform Infrared Spectroscopy (FTIR) analysis of prepared EA NPs and Amylase-EA NPs: The FTIR peaks of prepared EA NPs by using desolvation method\textsuperscript{13,15} before immobilization of amylase were observed at 3364 cm\textsuperscript{-1} that assigned to the possible stretching vibration of OH [amide A, mainly NH stretching vibration]; at 2920 cm\textsuperscript{-1} that approximate assigned C–H and C–H (methoxy compounds) stretching vibration; at 1643 cm\textsuperscript{-1} that assigned to having approximate vibration of (NH) C=O group (Figure-5). The FTIR peaks of EA NPs after immobilization of amylase were observed at 1747 cm\textsuperscript{-1} that assigned amide I (mainly C=O stretching vibrations) (Figure-6). Observed FTIR peaks at 3364 cm\textsuperscript{-1}, at 2920 cm\textsuperscript{-1} and at 1643 cm\textsuperscript{-1} were only observed in EA NPs before immobilization only (Figure-5) which confirmed that immobilization of amylase was done at those assigned activated functional groups (Figure-6). These observations were found to be very close to with previous observed FTIR analysis of nanoparticles\textsuperscript{6,9,16-19}.

The FTIR peaks of EA NPs by using emulsification method\textsuperscript{13,14} before immobilization of amylase were observed at 3313 cm\textsuperscript{-1} that assigned to approximate stretching vibration of OH [amide A, mainly NH stretching vibration]; at 1635 cm\textsuperscript{-1} that assigned to having approximate vibration of (NH) C=O group; 1541 cm\textsuperscript{-1} that assigned approximate possible amide II (the coupling out phase of bending vibration of N-H and stretching vibration of C-N bands) (Figure-7). The FTIR peaks of EA NPs after immobilization of amylase were observed at 3309 cm\textsuperscript{-1} that assigned to have approximate possible stretching vibration of OH [amide A, mainly NH stretching vibration]; at 1635 cm\textsuperscript{-1} that assigned to having approximate vibration of (NH) C=O group (Figure-8). Observed FTIR peak at 1541 cm\textsuperscript{-1} was only observed in EA NPs before immobilization only (Figure-7) which confirmed that immobilization of amylase was done at those assigned activated functional group (Figure-8). And, these observed FTIR finding were found to be comparable with that of previous FTIR spectral observations of nanoparticles\textsuperscript{6,9,16-19}.

Conclusion

Hence, from this brief FTIR spectral analysis, it was concluded that BSA NPs and EA NPs were successfully fabricated very well by using process of desolvation as compared to emulsification method. As well as, loading of \textit{Cicer arietinum} amylase into activated BSA NPs and EA NPs was also carried out with the best ease of activated functional groups into BSA NPs and EA NPs by desolvation method as compared to emulsification method. So, present work may be helpful to possible designing the used modified fabricated formulations for the preparation of albumin nanoparticles and make it more feasible for the effective immobilization and loading of biological components such as enzyme, drug, antibody, peptide and hormone molecules etc. And, these advanced fabricated BSA NPs and EA NPs can be used for cost effective and biocompatible nonviral nanovehicle as a rational nontoxic and eco-friendly colloidal carrier for targeted delivery green method that can be used in safe therapeutic approaches.
Figure-5
FTIR spectra of prepared activated EA NPs by desolvation method before the immobilization of *Cicer arietinum* amylase

Figure-6
FTIR spectra of prepared activated EA NPs by desolvation method after the immobilization having loaded *Cicer arietinum* amylase (immobilization done by glutaraldehyde coupling)
Figure-7
FTIR spectra of prepared activated EA NPs by emulsification method before the immobilization of *Cicer arietinum* amylase

Figure-8
FTIR spectra of prepared activated EA NPs by emulsification method after the immobilization having loaded *Cicer arietinum* amylase (immobilization done by glutaraldehyde coupling)
References


