Preparation of Selected Metal Ion bound Albumin Nanoparticles

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***Abstract*-** Metal deficiency like Zinc and iron among pregnant women is a significant Global health concern especially in developing countries. About 17% of world population is affected by Zinc deficiency. Several nano systems have been studied in previous work to increase the bioavailability of micronutrients like zinc. This study is aimed to develop suitable nanoparticles that can be used in vitamin formulations as a carrier for zinc ion so that it can be highly bioavailable even using small amount of zinc. For this, BSA nanoparticles were produced in the size range of 100-200nm, and then zinc was bound with these prepared nanoparticles. After binding with zinc, the size of nanoparticles was about 600-800nm.By using characterization techniques, the results obtained showed that zinc binds with BSA nanoparticles effectively and by using this zinc bound Albumin nanoparticles in vitamin formulations, bioavailability of zinc can be enhanced in future studies.

I. Introduction

Zinc is a trace element found important applications in antioxidants, to increase immunity, in cancer treatment, to cure infections and growth impairment [1]. WHO in 2006 recommended Zinc compounds that are water soluble like Zn-gluconate and Zinc citrate to cure infant's diarrhea [2]. Different zinc compounds are used in food industry, among all these zinc compounds, Zinc citrate has promising characteristics like it has no odor, contains high Zinc content, sparingly water soluble and cost friendly [3]. Bioavailability of these Zinc compounds depends mainly on solubility and stability at gastric pH [4]. To increase the solubility and in turns Bioavailability, Zinc compounds are encapsulated by various nanoparticles [5].

To improve the efficiency of drug delivery and targeting at a particular cell type [6], colloidal carrier systems are used for encapsulation of drugs [7]. In biological systems, two major parameters are particularly important of colloidal carrier systems i-e their particle size [8] and narrow distribution [9]. Changing the particle size will help to increase the efficiency of drug delivery in different systems.

For accurate diagnosis and highly efficient delivery systems for drug, NPs are potentially important due to their high drug targeting ability and prolonged period in blood circulations [10]. Nanoparticle-based drug delivery is evolving as a prevailing approach in several disease [11]. Conditions, as illustrated by Zhang L, et al. that clinical approval of nanoparticle preparations for fungal infections, multiple sclerosis, hepatitis A, and end-stage renal disease [12]. But these nanomaterials have some side effects thus an alternative way to use biocompatible substances to form NPs [13].

Among all the colloidal carrier systems, the proteins particularly albumin are of particular importance because they are highly biocompatible and biodegradable, they are non-toxic and non-immunogenic [14]. Shrawan Laminchane et al. (2019) reviewed the versatile behavior of albumin, its use in drug delivery system and its specific targeting ability at some tissues and organs [15]. Similarly, in 2020 Monica Joshi et al. also reviewed albumin nanoparticles as a Nano carrier to transport drugs at pulmonary areas [13]. There are three different kind of albumin nanoparticles i-e Ovalbumin, Bovine serum Albumin and Human Serum albumin. Both serum Albumin i-e BSA and HSA have similar properties like high water solubility, longer half-life in blood plasma, Amino acid residues 583 in BSA and 585 in HAS and their molecular weight [16]. BSA is most commonly used due to many advantages like it is easily available, have strong ability to bind with ligands, high solubility and low cost [17]. Elmirah Karami et al. in 2019 reviewed Albumin as a Nano carrier, its different types and methods of production.

Nanoparticles enters to the bloodstream by means of ingestion, inhalation or even by skin contact. Emulsion method is often used to prepare albumin microspheres and Nano spheres [18]. The particle size is 0.5-100 micrometer obtained by this method. By using high power ultra-sonification, Nano spheres of about 100nm can be prepared . One of the serious disadvantages of emulsion method is that we can't remove dispersion agent completely from particle surface. For the complete removal it needs a large amount of organic solvent which makes it difficult. Yating S et al. had developed a method i-e micro fluid method to treat the tumor related diseases. Haw Luo et al. developed a modification in NAB technology. They form non-cross linking Albumin nanoparticles by using Fenton reaction and used them in Nano medicine but here is one limitation that NAB technology is time and energy consuming process [19]. Another modification was done in 2020 by Ting Gong et al. They had attempted to increase targeting ability of BSA nanoparticles with PA and these PAB were used to treat rheumatoid arthritis.

To prepare the nanoparticles from albumin, albumin may act as a nanocarrier, stabilizer, albumin-polymer conjugate, scaffold and template [19]. Albumin has been used to extend the half-life of drugs. Eperzan had formulated a first marketed "albumin fused peptide drug "albiglutide" for the treatment of type II diabetes. By using the albumin nanoparticles of below 200 nm, drugs can be delivered to targeted sites because these small nanoparticles have permeating and retention ability i-e EPR effect hence they will accumulate in tumors. Albumin nanoparticles also increase the drug solubility that is used for intravenous administration;

thus, albumin can be used as a solubilizer in place of cremophor which is toxic. Abraxane has used albumin as a carrier in nanoparticle formation of paclitaxel for treatment of breast cancer. Kim et al., has formulated albumin fused curcumin nanoparticles for the treatment of breast cancer. They used "albumin bound technology' [20]. To use albumin nanoparticles in clinical applications, it is necessary to develop such technologies which are more reliable and gives better quality nanoparticles [21]. Albumin was clinically applied successfully by "Abraxane" hence making it ideal carrier for drug delivery and bio imaging. Mona A. Hassan et al. in 2019 have used nanoparticles after coated with Zn- citrate to prevent the gene expression complications that arise in rats by using CCl_4.

II. MATERIALS AND METHODS

Bovine serum Albumin was used to prepare nanoparticles. An aqueous solution of Glutaraldehyde (8%) and Glucose were used as cross-linkers. Ethanol 8% were prepared and sodium hydroxide were obtained from sigma -Aldrich. All reagents and solvents were of analytical grade and used without further purification.

Preparation of BSA nanoparticles:

Bovine serum albumin (protein) nanoparticles were prepared by using "desolvation method" which is a well-known method reported before in the literature. Firstly, 100 mg of Bovine serum albumin that was in dry (powdered)form, dissolved in 1ml distilled water.0.1N NaOH was added in solution to adjust the pH of about 7.2 and then solution was subjected to stir for about 10 minutes at room temperature. Dropwise addition of 4ml ethanol was made at a rate of 1-1.5ml/min and then subsequent addition of 40 microliter (25%) Glutaraldehyde was performed under continuous stirring and it the solution was kept to stir for about 22 hours. The pellets formed by continuous stirring were centrifuged three times at 15000rpm for 20 min each. The first supernatant was stored for later identification purposes. The pellets were washed with deionized water after each centrifugation and then subjected to ultra-sonication for 20minutes. The product formed was then freeze dried and stored at 4 ^{o}C in dark.

Binding with zinc:

After the formation of BSA nanoparticles, attempts were made to bind these nanoparticles with zinc by following the adsorption mechanism as described in literature. A stock solution of 2mM zinc chloride was prepared. About 100 microliter of this stock solution was taken and mixed with 20mg solution of previously prepared BSA nanoparticles and total volume of the solution kept about 2ml by adding distilled water [22]. The solution was kept on stirring for about 12hrs and then subjected to centrifuge at 13000 rpm for 10min. The supernatant was separated and stored for later identification.

Characterization of Nanoparticles

Fourier transform infrared (FTIR) spectroscopy

The band intensities and frequencies in different regions of the spectrum for the BSA, BSA with cross linkers and BSA with metal bound were observed by using FT-IR spectroscopy [23]. Samples were freeze dried to make them in powder form. These samples were pressed to KBr disks and kept to scan at about 600-4000cm^{-1} (wavenumber) in a transmittance mode [24].

Ultraviolet Absorbance spectroscopy

The Ultraviolet- visible spectra were evaluated by using a Ultraviolet-visible spectrophotometer (U-2910 HITACHI spectrophotometer) ranges from 190-500nm in 1cm quartz cell. Dilutions were made for all the materials whose spectra will be analyzed. These spectra provide information about the quality of binding of BSA with zinc ion.

Particle Size analyzer or DLS spectroscopy;

The size of nanoparticles prepared previously was analyzed by using DLS technique. Temperature maintained during analysis was 25 ^{0}C and disposable cuvette were used for sampling. The mode of analysis in these studies was "size distribution by intensity". BSA nanoparticles with glutaraldehyde and glucose were analyzed before and after zinc binding.

III. RESULTS AND DISCUSSION

FTIR analysis:

The Infrared spectra of pure BSA, BSA nanoparticles with crosslinkers i-e Glucose and glutaraldehyde and metal bound BSA nanoparticles (Figure 1) describes that due to vibration of peptide bonds, there are number of amide bands appeared in spectra.

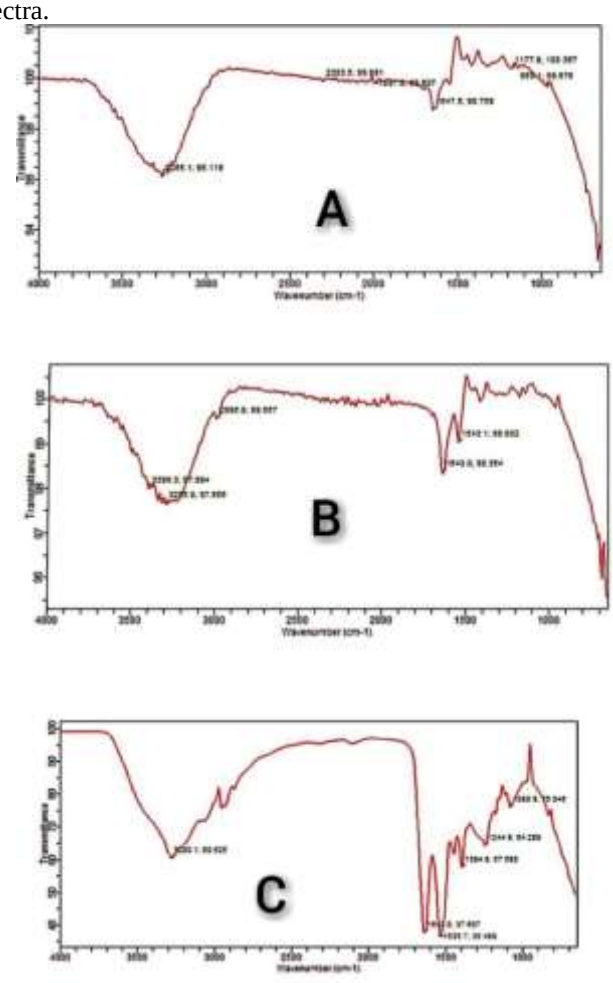

Figure 1 FTIR spectra of (A) pure BSA (B) BSA-Glutaraldehyde nanoparticles and (C) BSA-Glutaraldehyde zinc bound nanoparticles

As illustrated in Figure 1, there are four characteristic amide bands in the spectrum of pure BSA that includes Amide I band (Carbonyl stretching vibrations) at 1640cm^{-1}, Amide II band (coupling of N-H bend and C-N str) at 1535cm^{-1}, side chain and Amide III bands were at 1394cm^{-1} and 1244cm^{-1} respectively.
Spectra of BSA nanoparticles shows slight changes in Amide I, II and III bands as compared to the spectra of pure BSA. Cross-linking took place in the vicinity among all cross-linkers, according to analysis of FTIR and UV-Vis data [25]. With such a carrying capacity for drugs of 7.78%, produced nanoparticle were shown to have an 81.13% drug encapsulation effectiveness [26]. In addition, our release tests show that distribution of drug happens by concentration-dependent migration from the nanoparticle drug delivery system's matrix. These results show that including its was successfully encapsulated, with better solubility resulting in greater bioavailability. This nano formulation is made up of FDA-approved ingredients that might be used to encapsulate additional essential nutritious compounds.

UV analysis;

In the spectrum of Pure BSA without any crosslinkers, there are two considerable peaks. One is strong absorption around 208nm and the other is relatively weak at 280 nm. (Figure 2) After metal binding, the absorption peak at 278nm diminishes [27] to some extent and there is slightly blue shift occurs in wavelength. (Figure 3).

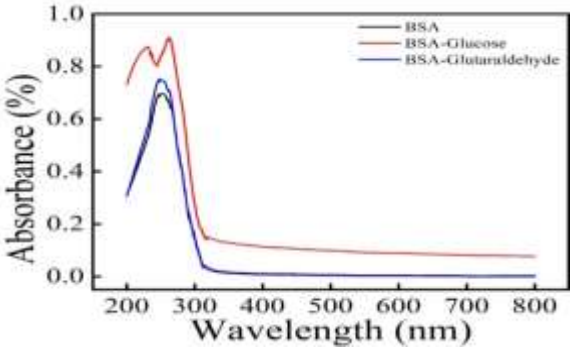

Figure 2 UV visible spectra of pure BSA, BSA with Glutaraldehyde and BSA with Glucose

DLS analysis:

The size obtained by DLS analysis for BSA- Glutaraldehyde nanoparticles was 178 nm and of BSA-Glucose nanoparticles was about 190nm. After binding with zinc, the size of these nanoparticles was 611nm and 868nm respectively (Figure 3).
The comparison of pure BSA and BSA nanoparticles spectra shows that there was a slight shift in absorption bands after the formation of nanoparticles. Along with these changes, there is small intensity changes in spectra that were caused due to binding with different groups of cross linkers [28].
After zinc binding, similar changes were observed mainly in Amide I and II band.

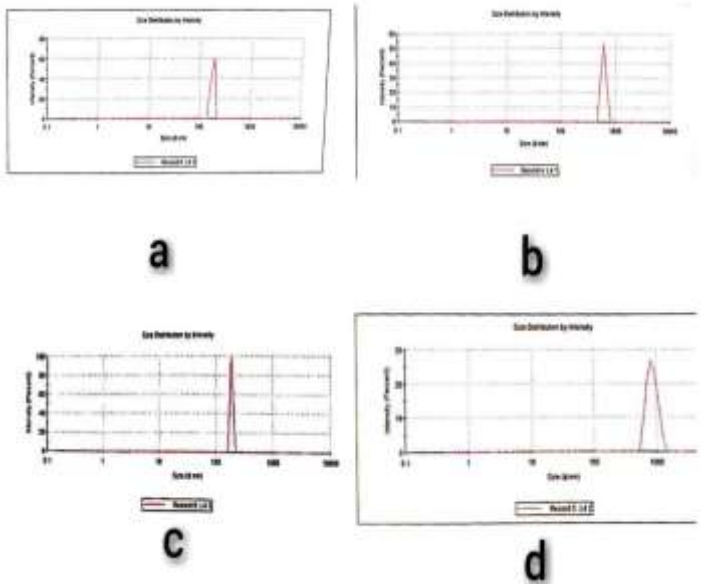

Figure 3 DLS spectra of (a) BSA-Glutaraldehyde (b) BSA-Glutaraldehyde zinc bound nanoparticles (c) BSA- Glucose nanoparticles and (d) BSA-Glucose nanoparticles with zinc bound

In the spectrum of Pure BSA without any crosslinkers, there are two considerable peaks. One is strong absorption peak around 208nm (peptide chain) and the other is relatively weak peak at 280 nm (disulphide bonds & aromatic residues) [29].
When nanoparticles of BSA formed, there was a slight change in intensity at 208nm and also some blue shift in wavelength was observed [30]. When these nanoparticles binds with zinc, the peak at 280nm diminishes that shows there is some involvement of disulphide bonds with zinc binding [31].

III. CONCLUSION

This study aims that zinc binding with BSA nanoparticles by using adsorption technique provide zinc that is highly bioavailable in human body in comparison with zinc salts. For this, Desolvation method was developed to form BSA nanoparticles. Ethanol was added as desolvating agent two different crosslinkers were used i-e glutaraldehyde and glucose and the results obtained were almost similar. Spectroscopic studies like FTIR, UV and DLS were performed indicating that nanoparticles are highly stable and are monodisperse.
Given the good data of results of this study, it can be a building block in future studies in which other zinc compounds can bind with protein nanoparticles so that their bioavailability in vitamin formulation can be enhanced and the deficiency of zinc can be resolved. of research work on open front. Generally all International Journals are governed by an Intellectual body and they select the most suitable paper for publishing after a thorough analysis of submitted paper. Selected paper get published (online and printed) in their periodicals and get indexed by number of sources.

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