International Journal of Pharmacy and Biological Sciences
ISSN: 2321-3272 (Print), ISSN: 2230-7605 (Online)
IJPBS™ | Volume 8 | Issue 4 | OCT-DEC | 2018 | 77-84
Research Article |Pharmaceutical Sciences | Open Access | MCI Approved| ज्ञान-विज्ञान विमुक्तये
|UGC Approved Journal |

BIOSYNTHESIS OF NANOSILVER AND ITS EFFECT ON THE INSOLUBILITY OF SIMVASTATIN

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ABSTRACT

Aim: *Metal nanoparticle-drug interaction is indeed merely explored area of research and guarantees some effective outcomes pertaining to improvement in biopharmaceutical characteristics essential for its therapeutic value. The present investigation was performed with the end objective of solubility enhancement of Simvastatin by a novel technique.* ***Methods*** *- The present investigation reported a green method for synthesis of nanosilver (NS) using Coriandrum sativum (CS) leaf extract. Synthesized NS were subsequently evaluated for its effect on the insolubility of Simvastatin. Synthesized NS were evaluated by visual observations, UV-Visible spectroscopy, Transmission Electron Microscopy, IR spectroscopy, zeta potential, and phase solubility study.* ***Results*** *- Color transformation was observed as an indication of the formation of NS. UV-Visible spectroscopic analysis revealed surface plasmon resonance at 437nm. TEM of NS showed spherical shape and size distribution was significantly in the range of 150 to 200 nm. SAED pattern showed diffused rings pattern. IR supports the evidence of the interaction favorable to enhance the adsorption of Simvastatin on NS. Zeta potential study also supports the evidence of adsorption phenomenon. Phase solubility study was carried out in and off a presence of NS which revealed that an increase in the concentration of NS proportionately increases the solubility of Simvastatin.* ***Conclusion*** *- Various drugs may have the potential to get adsorbed on NS / Silver nanoparticles, thus enhancing their biopharmaceutical characteristic. Present investigation supports the preliminary pieces of evidence which showed, the solubility of Simvastatin may be enhanced by adsorption phenomenon using silver nanoparticles.*

KEY WORDS

solubility enhancement, silver nanoparticles, Simvastatin

INTRODUCTION

According to Biopharmaceutical Classification system, Simvastatin belongs to Class II drug list having low solubility and high permeability. Its bioavailability is approximately 3-5%. Its log P value is 4.68, means highly lipophilic drug. it is insoluble in water practically 0.0013-0.0015 mg/ml at 23°C, so it is poorly absorbed from GIT and absorption is dissolution rate limited. It shows high variability in pharmacological effects [1]. About 40% of drugs not soluble in water in practice [3] and simvastatin belong to same category. In 2017 the global cholesterol-lowering industry was worth $19.2 Billion with forecast to grow 4.9% each year during the next five years which means that the industry will be worth $24.4 Billion in 2022[2], out of which, market share of simvastatin in 2013 was 36.4 % [4] and expected to remain same by the 2022. This means projected market worth of simvastatin may be $8.784 Billion by the year 2022. Simvastatin's biological half-life and bioavailability are 3 h and 5% indicating extensive

first pass metabolism in liver, respectively [3]. Correlating market worth of simvastatin with its bioavailability gives the strange truth that $8.3448 Billion worth simvastatin may unavailable biologically causing various side effects in the patients, and, just a mere, $0.4392 Billion of simvastatin will be bioavailable. Therefore, it is essential to augment its aqueous solubility, dissolution rate and bioavailability of simvastatin from its formulations.

The application of nanotechnology in life sciences, called as nanobiotechnology. It is already having an impact on diagnostics and drug delivery/targeting [6]. Silver and gold nanoparticles are the most important leading nanomaterials, providing an outstanding platform in bioresearch and biomedical applications [7]. It is needed to maximize the therapeutic effects mutually with a minimization of the undesired secondary ones. The application of noble metal nanoparticles as drugs nanocarriers presents two major advantages, first, they are able to transport several therapeutic molecules adsorbed on their exterior surface and second, owing to the presence of Localized Surface Plasmon Resonances, an enhancement of the spectroscopic signals of the molecules carried is produced thus permitting to observe them in their traverse through the body to the specific disease tissues [5]. Present study emphasizes on the green method driven synthesis of nanosilver (NS) and its subsequent effect on solubility of Simvastatin.

MATERIALS AND METHODS

Extraction of CS leaves and synthesis of nanosilver (NS)

NS were prepared by using Coriandrum sativum (CS) leaf extract. CS leaves were purchased from local market of Satara, India. The authentication of CS leaves was done from Department of Botany, Yashwantrao Chavan Institute of Science Satara (Maharashtra). The fresh leaves of CS were washed thoroughly with double distilled water in order to remove the dust and foreign material from the surface then air dried under shade at room temperature. The air-dried plant material was coarse powdered. Plant extract was prepared by boiling 10g of air-dried powder with 100 ml deionized water for 10 minutes. It was filtered using Whatmann filter paper No.1 and filtrate was directly used as green source for synthesis of NS. The $AgNO_3$ used in reaction was analytical grade chemical obtained from Sigma Aldrich and used without further purification. The source of silver was silver nitrate ($AgNO_3$) in distilled water. 1mM silver nitrate solution was prepared by adding 0.1699 g of $AgNO_3$ to 1L distilled water. In the single step green synthesis, 1 ml of CS leaf extract was added to 99 ml of 1 mM aqueous silver nitrate solution and autoclaved at 15 psi at 121^{o}C for 5 min [8]. Solution of same concentration was also kept at room temperature without autoclaving.

UV-Visible Spectral Analysis

Silver nanoparticles were formed by reduction of silver ion; it was monitored by measuring the absorption spectra in the wavelength range of 200-900 nm using Shimadzu UV-1700 Spectrophotometer. The spectrum was recorded and the maximum absorption wavelength was determined [9].

Particle Size Analysis [10]

The mean particle size of the silver nanoparticle was determined by using a Zetasizer. This analytical technique measures the mean diameter of the particle at 25°C, and at an angle of 90 degree (n=10). (Particulate system nano plus).

Zeta potential:

The zeta potential of the synthesized silver nanoparticle and conjugate [11] was measured by using zetasizer at 25°C (Particulate system nanoplus) using base as a water.

Transmission electron microscopy

The size and shape of nanoparticles were analyzed using transmission electron micrographs [12]. Particle morphology, size and shape were studied using TEM. SAED pattern was used to identify crystal structures.

FTIR study

FTIR measurements were carried out to identify the biomolecules responsible for capping and stabilization of metal nanoparticles synthesised. IR study [9] was carried out to check purity of drug. It was determined by Fourier Transform Infrared spectrophotometer (FTIR, Alpha, Bruker). The sample was scanned over wavelength region of 4000 to 500 cm-1 at resolution of 500 cm-1 by dispersing sample.

Phase solubility studies of Simavastatin

Excess amounts of simvastatin was added to 10 ml tubes containing aqueous solutions of increasing concentrations of AgNps and shaked at room temperature. At the equilibrium after 72 h, an aliquot from each vial was filtered by a syringe equipped with a Gellman Science Acrodisc® LC PVDF 45m filter. A portion of the sample was adequately diluted and analyzed by

spectrophotometry at 238 nm [13]. The experiments were carried out in triplicate.

RESULTS AND DISCUSSIONS

Biosynthesis of NS

Visual observation

The formation of silver nanoparticles was monitored with color change. After addition of CS leaf extract to silver nitrate solution, color was changed from transparent to yellow within 10 min. and after autoclaving it became reddish brown which indicated the generation of NS, this color changes arises because of the excitation of Surface Plasmon Resonance (SPR). Results were shown in Figure 1.

A B C

Fig. 1: A, Before autoclave. B, NS with extract without autoclave. C, NS with extract after autoclaving.

UV- visible spectroscopy

The UV absorption spectrum of synthesized NS has shown single peak at 437 nm. Spectrum was recorded for solutions with and without autoclaving. Although solutions without autoclaving develop a SPR peak which was steadily increasing in intensity, long term stability was not optimum. Solutions with autoclaving shown more symmetric peak and found more stable up to 10 months. SPR peak were recorded periodically to determine the stability of NS. The results were shown in Figure 2.

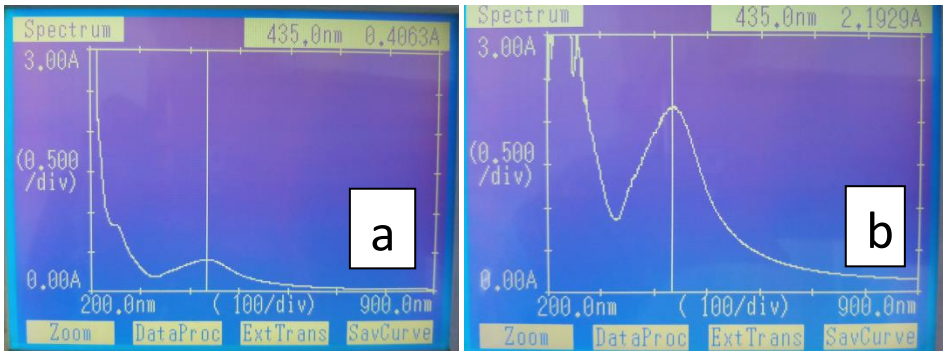

Fig. 2: UV visible absorption peak a: without autoclaving, b: with autoclaving

Particle size analysis

Polydispersity index (PI), also known as the heterogeneity index [15] was determined by the cumulants method at 25°C. The PI is a dimensionless number indicating the width of the size distribution and has a value between 0 and 1, being 0 for monodisperse particles [14]. The term "polydispersity" (or "dispersity" as recommended by IUPAC) is used to describe the

degree of non-uniformity of a size distribution of particles. PDI values bigger than 0.7 indicate that the sample has a very broad particle size distribution and is probably not suitable. In drug delivery applications a PDI of 0.3 and below is considered to be acceptable and indicates a homogenous population [15]. The average particle size was found to be 176.6 nm which confirmed the silver ions were reduced into nanoparticles. The polydispersity index (PI) of the silver nanoparticle was found to be 0.214; which although indicates the broad distribution but was homogeneous. The results of which were shown in Figure No. 3.

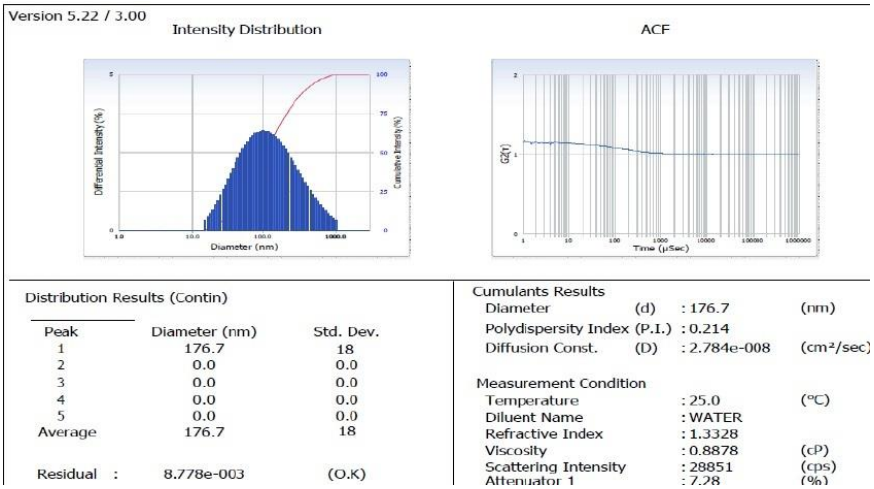

Fig. 3: Particle size analysis

Zeta potential:

Zeta potential analysis is a technique for determining the surface charge of nanoparticles in solution. High zeta potential (± 30 mV) leads to monodispersity. Low zeta potential (± 5 mV) can lead to agglomeration [17, 18]. The zeta potential of synthesized silver nanoparticles was determined by using zetasizer at 25°C, it was found to be +30.71 mV which indicated the mono dispersity of AgNps. The results of which were shown in Figure 4. Nanoparticles with positive zeta potential has greatest pharmaceutical significance. The attachment of nanoparticles to cell membrane seems to be most affected by the surface charge of the cell and nano particles. Cellular surfaces are dominated by negatively charged sulphated proteoglycans molecules [16]. Negatively charged particles can be rapidly opsonized and massively cleared by fixed macrophages. It is well known that the reticuloendothelial system (RES), mainly the liver and spleen, is a major obstacle to active targeting because of its ability to recognize these systems, remove them from systemic circulation, and consequently, avoid the effective delivery of the nano drug to organs other than those of the RES. Surface charge modification (especially positive) of nanoparticulate systems is the most common way to control the opsonization process.

Nanoparticles with positive zeta can also have a positive effect on skin drug delivery systems, since the skin carriers a negative surface charge due to carbohydrates [20] and phosphatidyl choline found in mammalian cells and contain negatively charged groups [19, 21].

Transportation of nanoparticles from the BBB is involving electrostatic interaction between a positively charged ligand and the negatively charged membrane of cells at the BBB that is named adsorptive mediated endocytosis (AME) [22].

Various tumor cells have elevated surface levels of negatively charged phospholipids. Negatively charged phosphatidylserine (PS), a constituent of the inner layer of human cytoplasmic membranes, can be translocated to the surface of cells during loss of membrane asymmetry [23].

The transfection efficiency of gene vector depends on the particle size and zeta potential. Because many proteins, DNA and cell membrane surface are slightly anionic, so a positive Zeta of nanopartilce not only has benefits for enhanced DNA loading efficiency, also might provide the effective accumulation in the target cells [24].

Since the negatively charged corneal surface [25], Positive ZP is important for ocular drug delivery since it can facilitate effective adhesion to the cornea epithelial surface, prolonging the drug release and enhancing the

drug bioavailability in the internal tissue of the eye [24]. Nanoparticles synthesized in present investigation gains positive zeta potential and may be used in several modified, targeted and advanced drug delivery systems.

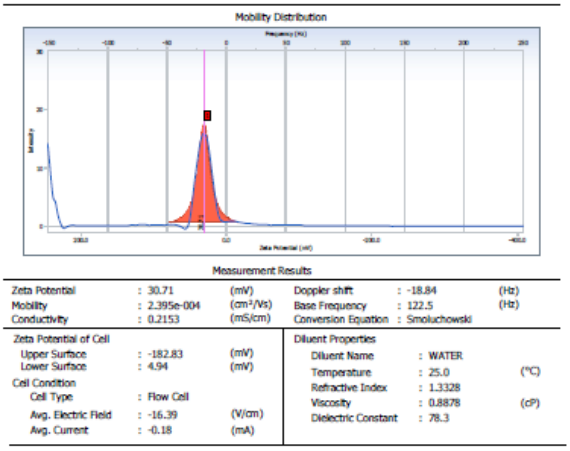

Fig. 4: Zeta potential

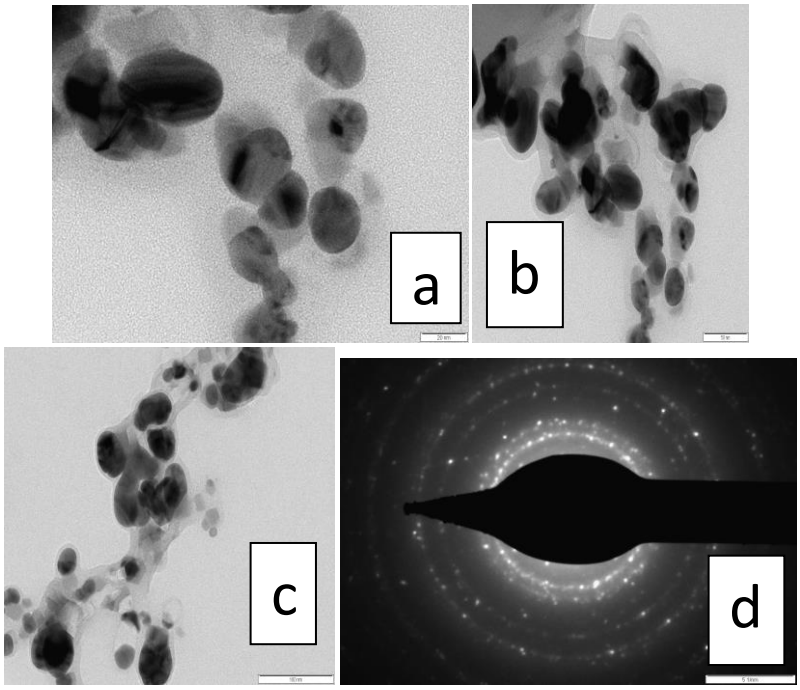

Fig. 5: TEM of (a): on scale of 20nm, (b) on scale of 50nm, (c) on scale of 100nm, (d) SAED pattern

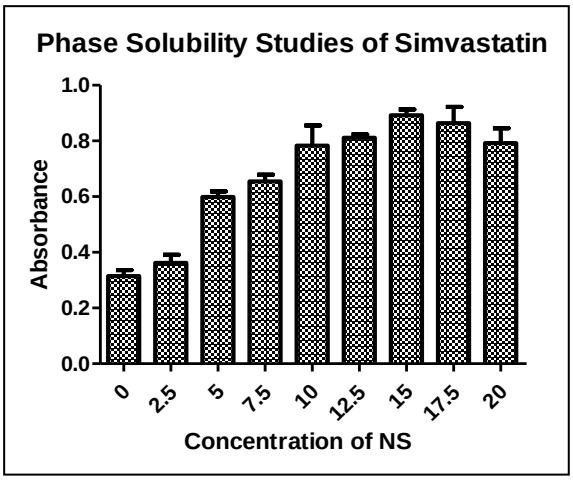

Fig. 6: Graphical presentation of phase solubility

Transmission Electron Microscopy

Figure 5 shows TEM images of prepared silver nanoparticles at 20, 50, 100 nm scale and SAED (selected area electron difraction suggested) pattern respectively, which shows that the prepared NS were evenly distributed in sample and were predominantly spherical in shape and the maximum particles size was within 150 to 200 nm. The selected area electron diffraction (SAED) suggested that NS were in polycrystalline nature, small spots making up rings [26].

Phase solubility study

Aqueous phase solubility of Simavastatin in presence of NS solution was carried out using shake flask method. It was observed that the solubility in presence NS was increased. Phase solubility study was conducted by preparing several saturated solutions of Simvastatin each containing NS in the concentration of 2.5ml more than the preceding solution. Solubility was proportionately increased up to 15 ml of Ag NPs solution. The results of which were shown in figure 6 and Table 1. Solutions containing more than 15 ml of NS were observed to have insignificant effect on the solubility. Zeta potential of saturated solutions containing more than 15 ml of NS were observed to have mere potential of + 7.7 mV which may cause the aggregation of NS and subsequently insignificant effect on solubility owing to decreased adsorption because of aggregation of NS. Increase in concentration of NS beyond 15ml may decrease the inter particulate distance and hydrogen bonding may mask the surface potential on the NS. The results of which were shown in Figure 7. Interaction of Simvastatiin with NS further confirmed by IR spectroscopy. IR spectra of Simvastatin, conjugate and NS shows all the characteristic peaks of Simvastatin were present in conjugate, therefore no significant interaction between drug and NS was observed except hydrogen bonding in conjugate. The results of which were shown in Figure 8. This adsorption type interaction of drug with NS [5] may be responsible to enhance the solubility of drug.

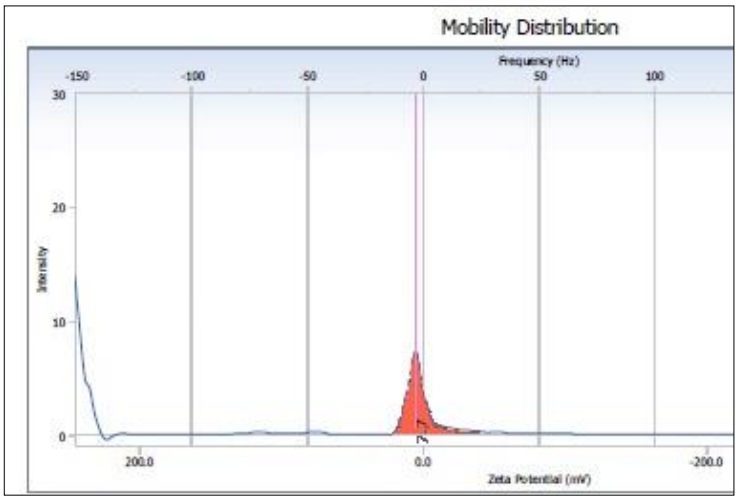

Fig. 7: zeta potential of solution containg 17.5ml of NS

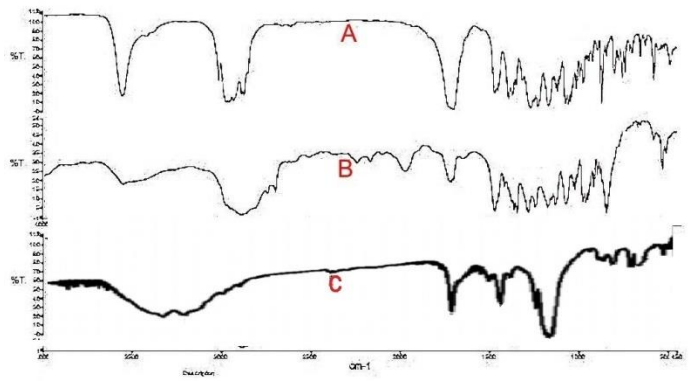

Fig. 8: IR spectrum, A, Simvastatin, B, conjugate, C, Nanosilver

Table 1: Phase solubility of Simavastatin:

Sr.No	Concentration of product in (ml)	Absorbance		Concentration of product in (µg /ml)
		Average	SD	
1	0	0.315	0.020873	8.18
2	2.5	0.361733	0.029202	9.70
3	5	0.5988	0.020164	15.18
4	7.5	0.654633	0.023871	17.31
5	10	0.7833	0.072085	19.68
6	12.5	0.8112	0.011676	21.22
7	15	0.892067	0.021072	24.94
8	17.5	0.863767	0.057951	23.05
9	20	0.792467	0.052671	19.37

CONCLUSION:

Various drugs may have the potential to get adsorbed on nanosilver (NS) / Silver nanoparticles, thus enhancing their biopharmaceutical characteristic. Present investigation supports the preliminary pieces of evidence which showed, the solubility of Simvastatin may be enhanced by adsorption phenomenon using NS.

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Received:06.08.18, Accepted: 08.09.18, Published:01.10.2018

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