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Research Article

Biocompatibility of Silver Nano Bio-conjugates from *Vitis vinifera* Seeds and its Major Component Resveratrol

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ABSTRACT

The study focused on the green synthesis of silver nanobioconjugates (AgNPs) from phenolic-rich fruit source, *Vitis vinifera* seed extract and its major component phenolic, resveratrol respectively. Sunlight exposure for 20 minutes was the method of choice for the synthesis of AgNPs of the extract as well as the phenolic, resveratrol. The synthesized nanobioconjugates were characterized using UV-Visible spectroscopy, Transmission electron microscopy (TEM), Energy dispersive X-ray analysis (EDAX), X-ray diffraction (XRD), Polydispersity index, Zeta potential and Fourier transform infrared spectroscopy (FTIR). The reduction of silver ions was confirmed by UV-visible spectroscopy with peaks at 440nm for both nanobioconjugates synthesized from seed extract and compound. The nanobioconjugates showed the spherical in shape with 14-35nm in size and crystalline in nature. The conjugates are well dispersed with 0.301 and 0.287 polydispersity index and the zeta potential range at -13.6 and -14.3mV for stability. The FTRI data proved that the components in grape seeds act as good reductants and stabilizers for the silver nanobioconjugate synthesis. All the synthesized nanobioconjugates exhibited steady and sustained release of the medicinal components conjugated, proving their druggability, and were biocompatible with human cells, demonstrating their safety. The findings of the study validate the anticancer properties of silver nanobioconjugates of *Vitis vinifera* and its active component resveratrol.

Keywords: Vitis vinifera, resveratrol, druggability, biocompatibility.

INTRODUCTION

Over the past years, nanotechnology has emerged as a promising strategy to resolve the technological impasses incurred in the diverse sectors of chemistry, medicine, environment, energy, agriculture, heavy metal industry, consumer goods and information technology. During the last few years, much research work has been carried out in the development of green synthesis methods for nanoparticles. Using the green synthesis approaches, around 200 plants belonging to different families have been screened for their potential effect and ability in the synthesis of silver, gold, copper, palladium and iron nanoparticles[1,2]. Nanoparticles possess a variety of shapes and they have been named based on their shape characterization as nanospheres, nanotubes, nanorods, nanoclusters or nanotriangles, which have applications in various fields. Controlling the morphology of nanoparticles is a most important process to manipulate nanoparticles in various zones of application[3].

The important advantages of nanoparticles include increased solubility and low production cost, resulting in rapid commercialization for medical purpose, especially in drug delivery[4]. A number of metal nanoparticles have been developed for the treatment of human diseases, which include cisplatin and its analogues to treat a variety of solid tumor cancers, silver compounds that are effective for infectious diseases and gold, used clinically for arthritis[5]. The properties of nanoparticles can be altered by surface modification and the distribution of nanoparticles depends on the surface characteristics. Depending on the particle surface chemistry, reactive groups on a particle surface will certainly modify the biological effects, whereas, different types and concentrations of surfactants have also been shown to change their distribution in the body and their effects on the biological systems significantly[6].

Recent research is focussing on herbal-oriented nanoparticles synthesis, with variability in the morphological, physical and chemical nature of nanostructures, which, in turn, are mediated and controlled by the various metabolites present in the plants and plant-derived products[7]. Secondary metabolites present in the plants are chemically very diverse in exhibiting many biological functions, which facilitate the synthesis of nanoparticles rapidly and by ecofriendly means[8,9]. In this regard, photochemical methods have recently aroused much attention because of the controlled reduction of metal ions with less reducing agent in the presence of light. Various studies have confirmed the formation of silver nanoparticles under simulated sunlight exposure in the presence of natural organic matter[10-13].

Grapes (*Vitis vinifera*) is one of the world most important cultivators, which has been consumed in various form as wine, juice, jam and pudding. Resveratrol, a polyphenolic phytoalexin present in the skin, seeds of grapes and other

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fruits demonstrates a broad range of biological activities[14,15]. Various researchers reported the use of resveratrol treatment in combating diseases such as cancer, cardiovascular diseases, diabetes, Alzheimer's disease, atherosclerosis, anti-inflammatory and age-related factors[16,17]. Earlier study conducted in our research group using grape fruit against oxidative stress-induced apoptosis in *Saccharomyces cerevisiae* cells, showed that the extract exhibited protective effect. With this background our study was formulated to synthesize and characterize the silver nanobioconjugates from *Vitis vinifera* extract and its active component, resveratrol.

MATERIALS AND METHODS

Preparation of the fruit extract

Samples of *Vitis vinifera* (pink variety grapes) were collected from the market of Coimbatore. The seeds were dissected and collected separately from the fruits. Then seeds were macerated with 50ml of methanol and kept in ultrasonic bath for 30minutes, and centrifuged at 5000rp for 20minutes. The extracts were used for the synthesis of silver nanobioconjugates. The major phenolic compound (resveratrol) of grapes were also involved in the synthesis of nanoparticles, which was purchased from MP, Biomedicals.

Synthesis of silver nanobioconjugates

The silver nanobioconjugates was prepared by adding 10ml of methanolic extract [grape seed extract] or 10ml of diluted purified compound (100µg resveratrol) to 90ml of 1mM silver nitrate (Sigma) solution. Based on the pilot study conducted in our laboratory the sunlight exposure method has been selected as the method of choice for the synthesis of silver nanobioconjugates. The mixture of extract or compound and silver nitrate solution was exposed for durations of 20 minutes. The mixtures were left undisturbed in the dark for 24 hours, after which the silver nanobioconjugates obtained were collected by centrifuging at 18,000 rpm for 20 minutes under refrigeration and washed three times with deionized water. The pellet was transferred to a pre-weighed container and dried in a hot air oven at 50ºC, and used for the further experiments.

Characterization of silver nanobioconjugates

The synthesized silver nanobioconjugates initially evaluated by UV-visible absorption spectroscopy based on the optical properties. A volume of 100µl of synthesized AgNPs was diluted with 900µl distilled water and subjected to spectral analysis in the wavelength range of 220nm - 800nm using nanophotometer (Shimadzu-Bio Spec-nano, Japan).

Transmission electron microscope is ideal for investigating nanomaterials, using very high resolution[18]. To determine the shape and size of the silver nanobioconjugates, the synthesized silver nanobioconjugates were subjected to TEM observation. About 1ml of samples was diluted with 9ml of double distilled water and placed in an ultrasonic bath for 10 minutes prior to use. A drop of well dispersed sample was loaded on carbon coated grid and it was allowed to dry. TEM images were recorded with an accelerating voltage of 80kV. The images were captured and documented under various magnifications and the size distribution of the resulting nanoparticles was estimated using high TECNAI G^2 with FEI TECNAI software. The TEM images were obtained and the energy dispersive X- ray (TECNAI F30, Genesis Rev. 3.0 software) analyses were also performed to estimate the composition of the synthesized silver nanobioconjugates.

X-ray diffraction (XRD) is a rapid analytical technique primarily used for phase identification of a crystalline material and can provide information on unit cell dimensions[19]. For obtaining the XRD pattern, the prepared AgNP powder was placed on a glass microscopic slide and dried in a hot air oven at 50°C. The process was repeated to form a layer on the glass slide. The samples were dried and analyzed with the help of an XRD instrument (PANalytical, XPERT-PRO diffractrometer) with a Cu source at 1.5406 Å wavelength as X-ray source in thin film mode.

FTIR analysis was carried out to identify the functional groups involved in the synthesis of silver nanobioconjugates using FTIR spectroscopy (Sigma, Infinity). The FTIR spectrum was recorded both for the seed extract / compound and the AgNPs in order to identify the functional groups.

The stability of the synthesized silver nanobioconjugates was measured using Zeta-potential. Zeta-potential measurement was done with a Zetasizer Nano ZS (Malvern Instruments) in a disposable cell at 25°C, and the results were analyzed using Zetasizer 7.01 software.

Drug release profile

The dialysis bag was pretreated and conditioned before using it for the experiment. The desired length of the dialysis bag was cut and immersed in 0.2M sodium bicarbonate containing 0.01M EDTA, and kept in a boiling water bath for 15 minutes. Then, the bag was allowed to cool and washed thoroughly in distilled water. The bag was then subjected to boiling in 0.01M EDTA for 15 minutes and washed thoroughly in distilled water. The prepared dialysis bag was then stored in distilled water at 4ºC until further use.

The silver nanobioconjugates (10mg) were dispersed in 1ml of PBS and packed into the prepared dialysis bag. Then the packed bag was dialyzed in PBS to monitor the release of silver ions across the membrane, over a period of 48 hours, which was measured spectrophotometrically at 4400 nm (Shimadzu-Bio Spec-nano, Japan), at one hour intervals upto 48 hours.

Biocompatibility of AgNPs

The biocompatibility of the synthesized AgNPs was monitored *in vitro* in terms of the extent of hemolysis, morphological changes in erythrocytes and whole blood clotting. The protocol of collection and the use of healthy human volunteers was scrutinized and approved by the Institutional Human Ethics Committee (AUW/IHEC-14-15/XPD-08).

Extent of hemolysis

The uptake of nanoparticles into red blood cells is of great interest in nanotoxicology. Hemoglobin is one of the important and major blood protein in the human body. The experiment was carried out as described by He *et al.*[20] with slight modifications. Blood was drawn from healthy volunteers, who were not on any medications. The blood was transferred into tubes containing sodium citrate at a ratio of 9:1 (blood:anticoagulant) and mixed gently. Red blood cells were obtained by centrifugation for 20 minutes at 2000g. The supernatant plasma was discarded and the erythrocytes were washed thrice with saline to remove the adhered serum proteins. The collected cells were resuspended in saline at a proportion of 1:5 ratio (centrifuged erythrocytes:saline). From this mixture, 100µl of cells were transferred into 1ml sample (50 µg nanobioconjugates dispersed in saline) and incubated at 37ºC for 1 hour with mild shaking. After incubation, the cells were centrifuged at 3000g for 5 minutes to remove the debris and intact erythrocytes. The absorbance of the supernatant obtained was measured at 542 nm. The results were compared with the control samples, cells treated with saline as negative control and cells treated with water as positive control. The percent hemolysis was calculated as follows:

$$\text{Hemolysis (\%)} = \frac{\text{OD of test sample} - \text{OD }(-)\text{ control}}{\text{OD }(+)\text{ control} - \text{OD }(-)\text{ control}} \times 100$$

Morphological changes of red blood cells

Cell morphology is key to investigating the maintenance of normal cellular functions. In order to determine the effects of nanobioconjugates on the morphology of blood cells, 50µl of anticoagulant-treated human blood was incubated with the samples and saline respectively for 20 minutes at 37ºC. After incubation, the changes in the appearance of the blood cells were examined by microscope. Whole blood diluted with saline was considered as negative control and was compared with the samples. The morphology of the red blood cells was observed using an inverted microscope (Metzer, India).

Measurement of whole blood clotting

The hemolyzing red blood cells (RBC) that were not trapped in the clot (formed on the surface of clot) were used to measure the whole blood clotting. A higher absorbance value of hemoglobin indicates a slower clotting rate. Blood was drawn from healthy individuals and transferred into citrate-containing tube, as mentioned in the earlier experiment. The silver nanobioconjugates dispersed in saline (10µl) were added into a 24 well plate. Saline was added to individual wells as a negative control. The clotting reaction was activated by adding 2.5ml of $CaCl_2$ to 25ml of blood. An aliquot (100µl) of activated blood was transferred to the wells containing the sample or saline and stored at room temperature for 30 minutes. At the end of the incubation time, the supernatants were carefully aspirated and 3ml of distilled water was added to the clot and incubated undisturbed for 5 minutes. The blood cells were lysed with the addition of water, which released the hemoglobin, that was measured at 542 nm.

RESULTS AND DISCUSSION

Characterization of silver nanobioconjugates

The AgNPs was evaluated by UV-visible spectroscopy, which is a very important for the analysis of metal nanoparticles. Both the silver nanobioconjugates synthesized from grape seed extract (VAgNPs) and component, resveratrol (RAgNPs) showed the characteristic peak at 440nm, which is the characteristic of AgNPs (Figure 1).

TEM has become the preferred technique for the precise analysis of morphology and size, particularly for nanoparticles, and EDX is a further reliable tool to determine the elemental composition of the nanoparticles[21]. The nanobioconjugates synthesized from *Vitis vinifera* seed extract and their active compound (resveratrol) showed spherical shape (Figure 2-a,b). The size of the nanobioconjugates for extracts and compounds were 16-35nm and 14-28nm in size respectively. All of them are very well dispersed, without aggregation. The size of the nanobioconjugates fell well within the range of nanometer scale (≤100nm), which clearly showed that the bioconjugates synthesized were in nanoscale. The compositions of the synthesized silver nanoparticles were determined by EDX. The EDX profile of the nanobioconjugates synthesized from *Vitis vinifera* seed extract and their active compounds, resveratrol showed the presence of typical peaks of silver (Figure 2-c,d). Additional peaks were also observed which indicated the presence of carbon and oxygen, representing the existence of organic compounds in the silver nanobioconjugates.

The X-ray diffraction profiles of the synthesized silver nanobioconjugates are depicted in Figures 2 (f and g). All the silver nanobioconjugate profiles showed typical peaks corresponding to the face intensities of standard silver nanoparticles (Reference Pattern: Silver, 989-060-4629 (Figure 2-e) of four different peaks of 2θ values at 38.162, 44.313, 64.464, 77.424 that indexed to [111], [002], [022], [113] Braggs reflections of the cubic structure of silver. This confirmed the presence of silver in the nanobioconjugates and the highly crystalline nature of the particles. The pattern of nanobioconjugates synthesised from grape seed extract showed some additional peaks, which indicated the presence of organic molecules present in the extract. Thus, the results of X-ray diffraction corroborated with our results obtained in the EDX profile, which validated the presence of organic molecules that facilitate the synthesis of nanobioconjugates.

The stability of nanoparticles is analyzed through zeta potential. This potential is an indirect measure of the surface charge. The measurement of the zeta potential allows for predictions about the storage stability of colloidal dispersion[22]. The biosynthesised silver nanobioconjugates were studied for their stability and dispersion by zeta potential and poly dispersity index analysis respectively. The zeta potential values of the synthesised nanobioconjugates from *Vitis vinifera* seed extract and resveratrol were -13.6mV and -14.3mV respectively (Figure 3). These values were found to be well within the stable range (-20 to +20mV). There was a slight shift of the peaks to the negative side, indicating that the synthesised silver nanobioconjugates contain a net negative charge.

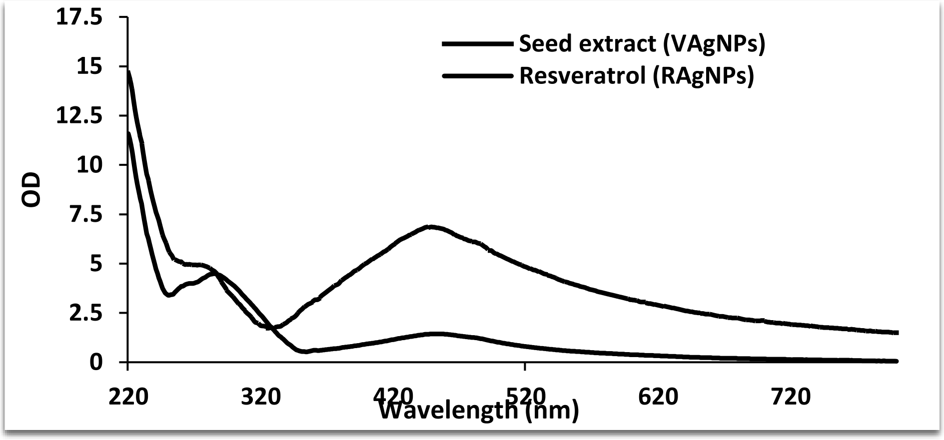
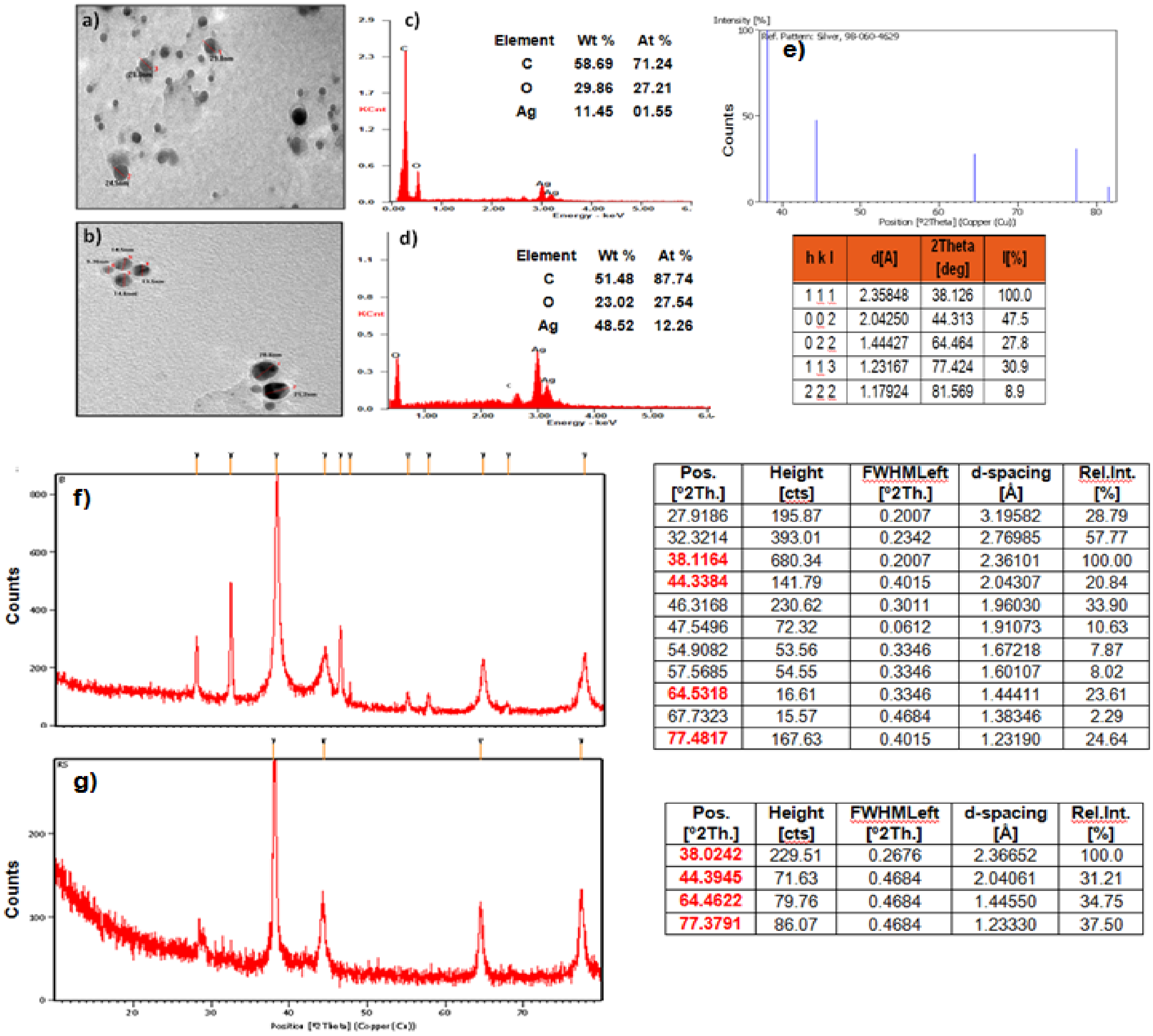


Figure 1: Absorption spectrum of silver nanobioconjugates



h k l	d[A]	2Theta [deg]	I[%]
1 1 1	2.35848	38.126	100.0
0 0 2	2.04250	44.313	47.5
0 2 2	1.44427	64.464	27.8
1 1 3	1.23167	77.424	30.9
2 2 2	1.17924	81.569	8.9

Pos. [°2Th.]	Height [cts]	FWHMLeft [°2Th.]	d-spacing [Å]	Rel.Int. [%]
27.9186	195.87	0.2007	3.19582	28.79
32.3214	393.01	0.2342	2.76985	57.77
38.1164	680.34	0.2007	2.36101	100.00
44.3384	141.79	0.4015	2.04307	20.84
46.3168	230.62	0.3011	1.96030	33.90
47.5496	72.32	0.0612	1.91073	10.63
54.9082	53.56	0.3346	1.67218	7.87
57.5685	54.55	0.3346	1.60107	8.02
64.5318	16.61	0.3346	1.44411	23.61
67.7323	15.57	0.4684	1.38346	2.29
77.4817	167.63	0.4015	1.23190	24.64

Pos. [°2Th.]	Height [cts]	FWHMLeft [°2Th.]	d-spacing [Å]	Rel.Int. [%]
38.0242	229.51	0.2676	2.36652	100.0
44.3945	71.63	0.4684	2.04061	31.21
64.4622	79.76	0.4684	1.44550	34.75
77.3791	86.07	0.4684	1.23330	37.50

Figure 2: TEM and EDAX pattern of VAgNP (a,c) RAgNP (b,d), XRD pattern of silver (e), XRD pattern of VAgNP (f) RAgNP (g).

Poly dispersity index of all the four nanobioconjugates recorded values were 0.301 and 0.287 for the nanobioconjugates fabricated from *Vitis vinifera* seed extract and resveratrol. These results clearly indicated that all the nanobioconjugates are well-dispersed without aggregation. Thus, our results showed that the nanobioconjugates of all the four biological test materials used in the present study, possessed good stability, were well dispersed and hence are expected to have long shelf-life. This study proposes that the silver nanobioconjugates

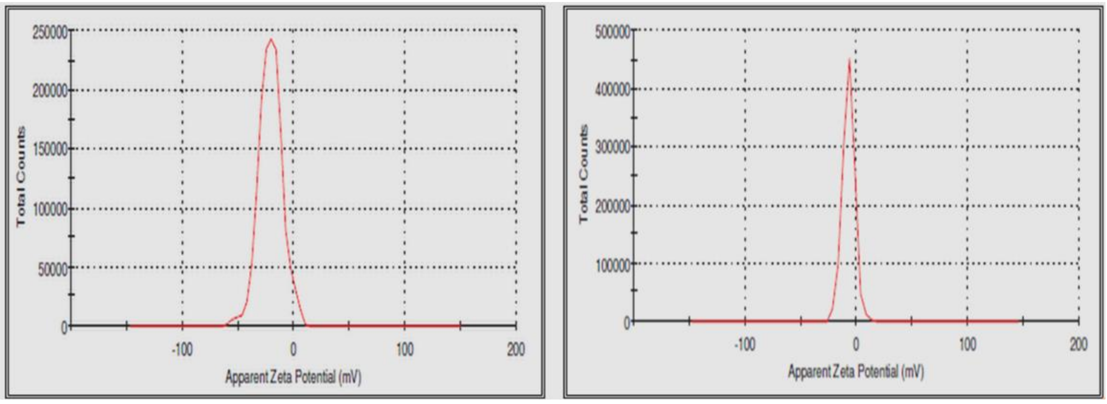


Figure 3: Zeta potential of VAgNP (a) RAgNP (b)

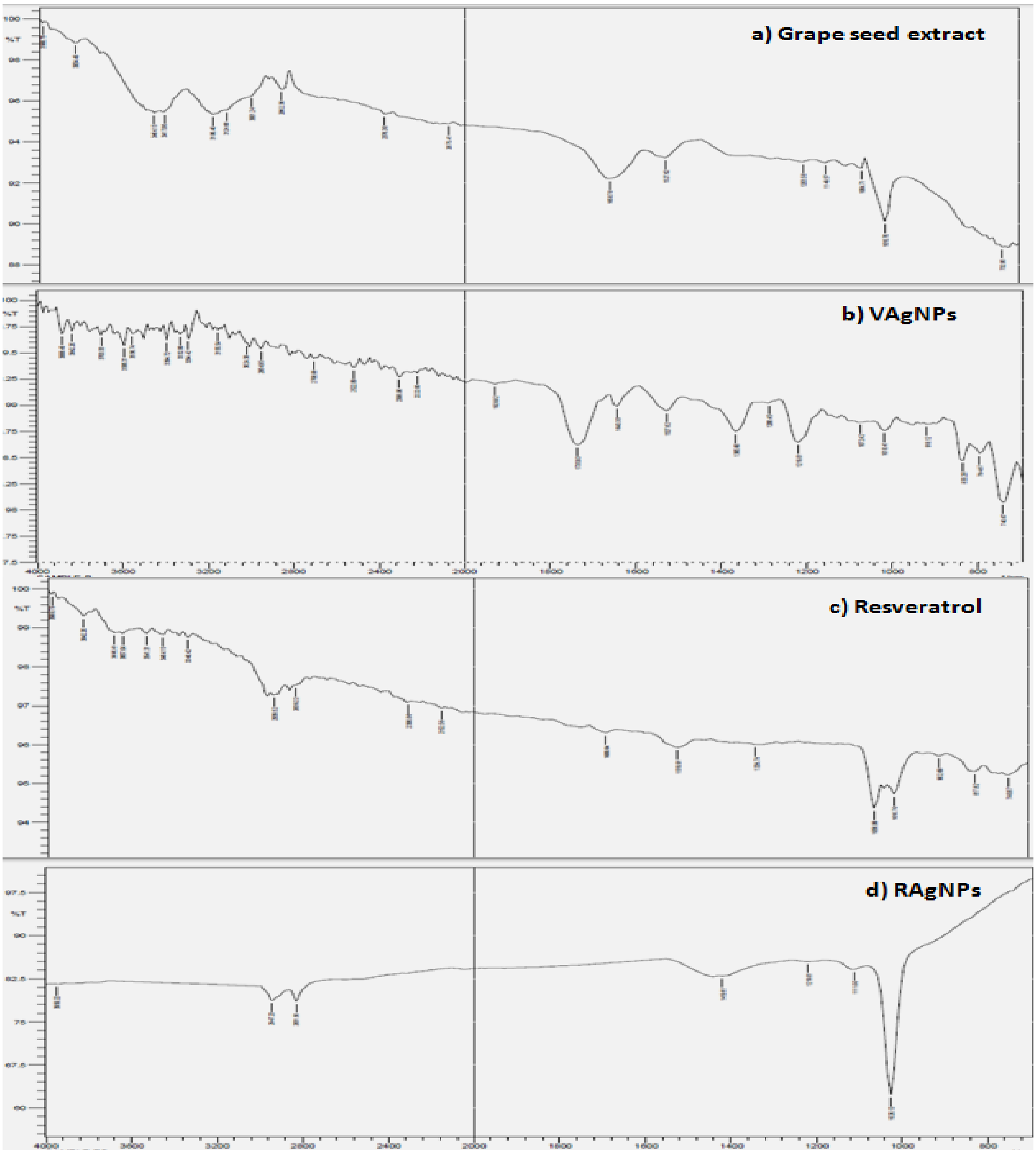


Figure 4: FTIR spectrum of silver nanobioconjugates

synthesized may have promising potential as drug delivery systems.
FTIR is an important key to identify the functional groups involved in the reduction of silver nitrate to silver nanobioconjugates and stabilization of conjugates[23]. The FTIR spectrum was analyzed and documented for the nanobioconjugates synthesized from the four different test materials as well as their non-nano sources (extract and compound). The FTIR spectrum of the nanobioconjugates synthesized from grape seed extract [Figure 4 (a, b)] showed functional groups of hydroxyl group (3484.15 cm^{-1}, 3417.86 cm^{-1}), alkanes (2862.36 cm^{-1}), alkenes (1658.78 cm^{-1}), nitro (1527.62 cm^{-1}) and aliphatic amines (1010.70 cm^{-1}), indicating that these groups may be engaged in the production of the silver nanobioconjugates. The spectrum of resveratrol (Figure 4 (c, d) confirmed the characteristic peaks of functional groups at 3356.14 cm^{-1} (OH group), 1635.64 cm^{-1} (aromatic ring) and 1512.19 cm^{-1} (aliphatic group) involved in the synthesis and stabilization of silver nanobioconjugates.
The peak patterns obtained with the silver nanobioconjugates of *Vitis vinifera* seed extract and resveratrol showed several similarities, indicating that they may share common features and functional groups.
Having characterized the formation of the ideally suited structures, their ability as drug carriers and biosafety were assessed. This was done by tracing the drug release profile of the synthesized nanobioconjugates and by testing their biocompatibility with human cells.

Drug release profile

The drug release profiles of the nanobioconjugates prepared from the extracts of the *Vitis vinifera* selected for analysis, were recorded from 0 hour upto 48 hours at one-hour intervals. The profiles showed that the nanobioconjugates released steadily upto 14 hours, after which a plateau of release was observed (Figure 5). This pattern suggested that the AgNPs synthesized could release the components in a steady and sustained manner, avoiding toxic spikes.
Following the drug release profile, the toxicity of silver nanobioconjugates synthesized from *Vitis vinifera* and resveratrol was tested on red blood cells from healthy human volunteers, to determine the biocompatibility. The extent of hemolysis for the synthesized silver nanobioconjugates from *Vitis vinifera* and resveratrol were found to be 0.126% and 0.58% respectively which was less than 5%. In contrast, 100% hemolysis was recorded in positive control (water). Thus, the results showed that the synthesized nanobioconjugates from the extracts and the compounds were not toxic to human red blood cells.
The non-toxicity of the synthesized AgNPs was futher investigated based on the changes in the morphology of human red blood cells administered with the silver nanobioconjugates. The morphology of the red blood cells was observed using an inverted microscope and the photomicrographs are presented in Figure 6.
Exposure of RBCs to water caused complete hemolysis, as observed by a totally disrupted cell morphology. The cells in this treatment group showed disorganized structures and loss of membrane integrity. On the other hand, the RBCs subjected to AgNPs treatment showed normal morphology of the cells, which was comparable to the saline treated group. These observations further proved that the AgNPs were absolutely biocompatible in nature, and are safe for human use.
The effect of silver nanobioconjugates from *Vitis vinifera* and resveratrol on whole blood clotting was recorded as a measure of toxicity. Both the silver nanobioconjugates showed no significant differences in blood clotting, when compared with saline control (Saline - 0.235 ± 0.02, VAgNP - 0.252 ± 0.01, RAgNP - 0.223 ± 0.01). This confirms that all the four nanobioconjugates effectively prevent cell clumping and clotting, reflecting their safety and biocompatibility.
Thus, all the above experiments revealed the sustained phytochemical release and confirmed the non-toxic nature of the synthesized silver nanobioconjugates. This detailed study articulated the high biocompatibility nature of silver nanobioconjugates synthesized from *Vitis vinifera* and resveratrol.

DISCUSSION

In the past decade, nanotechnology has shown potential and significant improvements of providing advanced materials for the future. Similar to bulk metals, the profound role of vacancies on the properties of nanomaterials has created curiosity in estimating their formation and characterization. A range of functionalities can be included within a nanoparticle complex, including surface chemistry that allows attachment of cell-specific ligands for targeted delivery, surface coatings to increase circulation times for enhanced bioavailability, specific materials on the surface or in the nanoparticle core that enable storage of a therapeutic until the target site is reached, and materials sensitive to local or remote actuation cues that allow controlled delivery of therapeutics to the target cells. All these characteristics are very essential in medical application, which can be determined by the chemical and physical nature of the nanoparticles[24].
The characteristic peak of grape seed extract mediated synthesis, found at 440 nm, which has also been reported by many researchers working with AgNPs. This absorption band was observed for AgNPs synthesised with *Mukia maderaspatana*, *Kedrostis foetidissima* and *Cayratia pedata*[25], seed extract of *Calendula officinalis*[26]. Similarly, nanoparticles synthesized using seed extract of *Vigna radiata*[27] and *Elettaria cardamomom* seed extract[28] showed a characteristic peak at 440nm.
Electron microscopy techniques such as transmission electron microscopy (TEM) can easily provide accurate size measurements with sub-nanometer resolution and aggregation[29]. The TEM images revealed both AgNPs, namely, those from seed extract and resveratrol to be spherical in shape and as monodispersed material, ranging in nanosize. Several researchers working on the synthesis of nanoparticles have used TEM extensively to characterize the morphology of particles. The nanoparticles synthesized from *Arabidopsis* plant extract

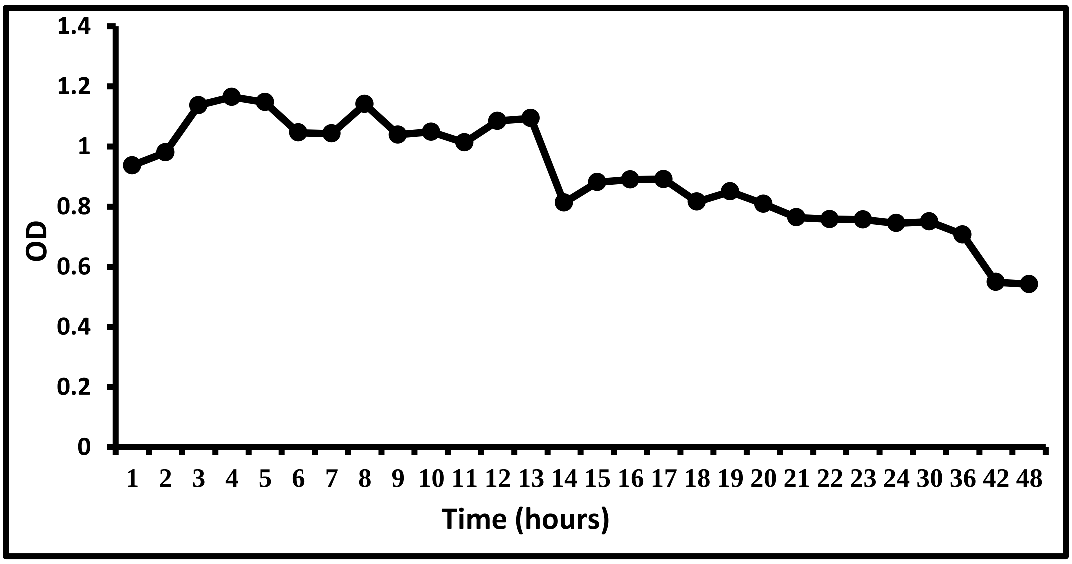


Figure 5: Drug-release profile of silver nanobioconjugates synthesized from *Vitis vinifera* seeds
The dotted line indicates the trend of release.

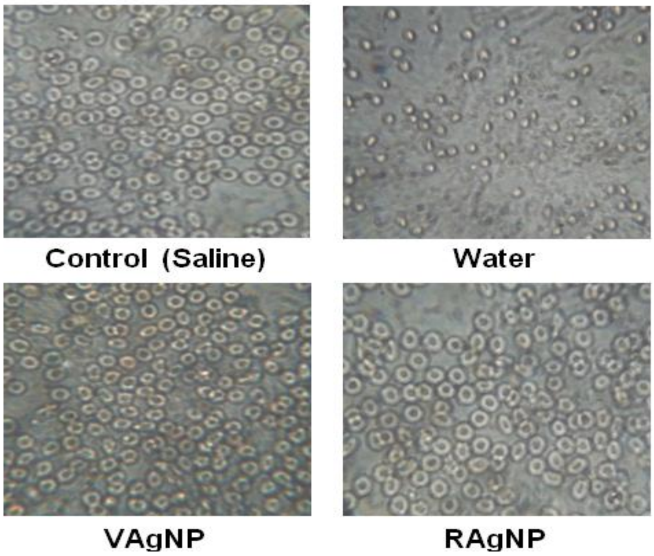


Figure 6: Effect of silver nanobioconjugates on the morphology of human blood cells

under sunlight exposure showed a particle size of 8-47 nm with spherical, triangular and decahedral in shapes[30]. The extract of *Erigeron annuus* (L.) pers flower extract formed spherical particles and were found to vary from 15 to 60 nm and 20 to 100 nm for AgNPs and AuNPs, respectively[31]. The EDX spectra of all the synthesized nanobioconjugates in our study showed the presence of carbon, oxygen and silver, which proved the conjugation of organic molecules in the synthesis AgNPs. Similar results have been reported by several researchers. The presence of carbon and oxygen in AgNPs of *Musa balbisiana* (banana), *Azadirachta indica* (neem) and *Ocimum tenuiflorum* (black tulsi)[32], aqueous extract of seaweed of *Turbinaria conoides*[33], aqueous extract of edible fruit of *P. peruviana*[34], Ceropegia *thwaitesii*[35], tea leaf extract[36].

In these study, the XRD patterns of the nanobioconjugates reaffirmed the presence of silver and organic material in the bioconjugates synthesised. They also showed the crystalline nature of the AgNPs. Several authors have used XRD to confirm the presence of silver and to determine the crystalline nature of the particles synthesized. The XRD of the silver nanoparticles synthesized from *Terminalia bellirica* fruit extract possessed inter-planar reflections of the cubic crystal system confirming the presence of silver[37], which was observable in our study also. Another researcher reported similar results with AgNPs synthesised from aqueous root extract of *Erythrina indica Lam* under room temperature, which showed the presence of silver[38]. Our results are in agreement with these reports. From the XRD spectra, the successful bioconjugation of all the test materials was confirmed, and the synthesized nanobioconjugates were shown to have crystalline nature.

The purpose of zeta potential measurement indicate the repulsive force that is present and can be used to predict the long-term stability of the product[39]. Various researches have exploited the importance of zeta potential in nanoparticle characterization. The biosynthesized silver nanostructures from *Terminalia chebula* extract showed a negative zeta potential with higher stability, which was suggested to be due to the capping of polyphenolic constituents present in the extract[40]. Qu *et al.* reported a highly negative zeta potential for silver nanoparticles synthesised from *Agrimoniae herba* extract[41]. The zeta potential of both the silver nanobioconjugates synthesized showed a negative value and fell well within the stability range.

Following the zeta potential measurements, FTIR analysis was carried out to determine the functional groups present in the extract and compounds contributing in the formation of silver nanoparticles. The FTIR spectrum of silver nanoparticles from leaf extract of *Nigella sativa* indicated the role of different functional groups in the synthetic process[42]. The FTIR spectrum of AgNPs of *Vinca rosea* highlighted the functional groups involved in the synthesis, such as amine, carboxylic acids and alkenes[43]. Murugan *et al.* reported the presence of different functional groups from alkane, methylene, alkene, amine and carboxylic acid present in *Artemisia vulgaris* leaves that may act as a stabilising agent in AgNPs[44]. Our results are in agreement with all the above studies showing the presence of several functional groups in *Vitis vinifera* seed extract and resveratrol, which were expressed in silver nanobioconjugates, possibly rendering capping and stabilization to the particles.

Thus, all the different parameters used in the study to characterize the AgNPs showed that there was successful conjugation between the organic phytocomponents and silver, to form well-dispersed, spherical nanobioconjugates, ranging in nanosize. There is an explosion of studies in nanomaterials in biological applications such as diagnostics, therapeutics and targeted drug delivery. This has raised concerns about the risk of usage of nanomaterials and human health and safety. Thus, it becomes imperative to study the efficacy of the nanobioconjugates in releasing their cargo in the human system, as well as their safety, in terms of biocompatibility to human cells.

The drug release observation showed that the silver nanobioconjugates synthesized in the present study could bring about sustained release of the phytocomponents. This strongly supports the druggability of the nanobioconjugates. An *in vitro* drug release study of AgNPs conjugated with azathioprine showed about two thirds of drug release within 24 hours period[45]. Bondarenko *et al.* observed the dissolution of casein coated AgNPs in test medium within 4 hours[46]. Similarly, Wang *et al*. reported that methotrexate was released from the methotrexate-silicon nanoparticles during the first 12 hours, followed by a sustained release up to 96 hours[47]. In this context, the observation made in the present study that silver nanobioconjugates showed a steady release, suggesting that this nanobioconjugates have a strong potential to contribute as a targeted drug delivery system, gains importance.

The biocompatibility of the AgNPs synthesized in the present study was tested in terms of the extent of hemolysis triggered, morphological changes to blood cells and coagulation pattern of whole blood. These are standard parameters directly indicative of toxicity of AgNPs. It is well acknowledged that, irrespective of the route of administration (oral or intravenous), silver nanoparticles enter the blood circulation and are then further distributed in the body[48]. Thus, the blood cells are the first systems to encounter the AgNPs entering the human system, and take the brunt of the toxicity, if any.

Several other studies have also been reported, wherein, lower toxicities were observed with the biologically synthesized nanoparticles than the chemically synthesized ones. The nanoparticles synthesized from *Euphorbia heterophylla* showed less toxicity to the human red blood cells, than the chemically synthesized silver nanoparticles[49]. Similarly, in another study, the silver nanoparticles synthesized from aloevera showed very low toxicity[50]. The alcoholic extract of tulsi leaf mediated silver nanoparticles also showed very low red blood cell lysis when compared with conventional drug[51]. The gold nanoparticles synthesised from aqueous extract of pods of *Peltophorum pterocarpum* revealed anticoagulant activity[52]. The silver nanoparticles synthesised from wheat bran xylan showed resistance in clotting period when compared with the blood without any anticoagulating agent[53]. With the background of these studies, the biocompatibility of silver nanobioconjugates from *Vitis vinifera* and resveratrol reveals that they were not toxic and act as very good biocompatible materials, which can be applied in the biomedical applications.

CONCLUSION

Thus, all the different parameters used in the study to synthesis and characterize the silver nanobioconjugates showed that there was a successful conjugation between the organic phytocomponents and silver, to form well-dispersed, spherical nanobioconjugates, ranging in nano size. The nanobioconjugates expressed good stability profile and a net negative charge on their surfaces. The presence of the functional groups on the surface of the conjugates was also confirmed, indicating their potential bioactivity. Having characterized the formation of the ideally suited structures, their ability as drug carries and biosafety were assessed. This was done by tracing the drug release profile of the synthesized nanobioconjugates and by testing their biocompatibility with human cells which reveals that they were not toxic and act as a very good biocompatible material, which can be applied in the biomedical applications.

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