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A STUDY ON BIOCOMPATIBILITY AND DRUG RELEASING PROFILE OF PEGYLATED SILVER NANOPARTICLES FROM *VOLKAMERIA INERMIS* LEAVES

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ABSTRACT

Silver Nanoparticles synthesis is an expanding area of research due to their properties and possible applications in several novel technologies. AgNPs are Essential in the biomedical field to diagnosis, treatments, mode of drug delivery and in bioremediation applications. The present research work was focussed on the biocompatible and the drug releasing nature of PEGylated silver nanoparticles (PEGylated AgNPS) synthesized from the ethanolic leaves extract of *Volkameria inermis*. The biocompatibility was studied in red blood cells collected from human healthy volunteers. In which, haemoglobin release analysis and the morphology of RBC was observed. The PEGylated AgNPS was found to be hemocompatible at the tested concentrations. Further, the cells does not show any morphological changes indicating the biocompatible nature. The drug release study followed at three different pH conditions i.e., pH 7.4, 6.8 and 5.5. The study resulted that PEGylated AgNPs showed an obvious pH related release behaviour. The drug release was found to be more rapid and sensitive at endosomal pH (pH 5.5). Hence, these nanoparticles are much biocompatible, environmentally safe and can be utilized in many medical applications.

KEYWORDS

Silver nanoparticles, *Volkameria inermis*, hemocompatible, cancer and Biocompatible.

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INTRODUCTION

Nanotechnology placed an emerging domain of medical science as it can be utilized in all research fields. Phytocompounds are valuable and encouraging candidates for synthesizing green silver nanoparticles (AgNPs) which possess great potentials toward chronic diseases[1]. Today the Nanoparticles and nanomaterials attempt a great

attention with highly attractive platform for a diverse array of biological applications. Nanoparticles are more predetermined treatments for difficult to manage diseases such as Oxidative diseases. Treatment of cancer is a biggest challenge to prevent non-cancerous cells from destruction. Current mode of treatment, either oral or parenteral, circulates in the entire body and cause harm[2]. Targeted drug therapies using nanosized for-mutations can be a useful approach to rectify this problem and only the proliferating cancerous cells will be targeted for cytotoxicity. Nanosized formulations are truly remarkable gift for the treatment of chronic disease such as cancer[3].

The Nanoparticle synthesis is one of the important biological methods with the help of Green Biotechnology. AgNPs from plant compound is one of the most accepted methods as it provides various advantages over conventional techniques from both chemical and physical methods. The nanoparticle synthesis from medicinal plant technique is eco-friendly, easy, no more sophisticated instruments and chemicals are required. Moreover the plant derived compounds are involved as reducing agents and stabilizing agents with no toxicity effects[4]. Ultimately reducing the overall cost of the formulation many scientists focused on herbal medicine[5,6]. Synthesis of nanoparticles from medicinal plant is another good alternative source for conventional methods of drug formulations. The drug development using this method is more stable with the desired shape and size[7,8]. Based on several evidence our research work is concentrated on Synthesis of PEGylated Silver nanoparticle.

In this research paper, the PEGylated AgNPs synthesized from the ethanolic leaves extract of *Volkameria inermis* was tested for its biocompatibility and its drug releasing sensitivity at different pH conditions. *Clerodendrum* is a medicinal valued plant which have high antioxidant potential and has been frequently voted as a traditional remedy which is used to treat against bronchitis, asthma, liver and stomach disorders. There are many classifications provided in Clerodendrum family and the common synonym is *Volkameria inermis* and it belongs to the family member of Lamiaceae with lot of medicinal properties since 1885[9]. Apart from these, several other types of mankind research are in pipeline with well-developed nanoparticles using the green synthesis method and their potential role in medical field.

MATERIAL AND METHODS

Collection of Plant Sample

Fresh leaves of *Volkameria inermis* (Figure No.1) were collected in an area free of pesticides and other contaminants from Tamil Nadu Agricultural University (TNAU), Coimbatore district and authentication was done at Botanical Survey, Tamil Nadu Agriculture University (TNAU) Coimbatore, India (BSI/SRC/5/23/2015/Tech/2082). The collected leaves were washed thoroughly in tap water, shade dried and powdered.

Preparation of Ethanolic Leaves Extract of *Volkameria inermis*

To 10g of powered leaf sample 100ml of ethanolic solvent was added (10g/100ml). Plugged with cotton wool and then kept on a rotary shaker at 190-220rpm for 24 hours. After 24 hours the extract was filtered and the filtrate was concentrated using flash evaporator and stored in air tight containers at 4°C and used for further experiments[10].

Synthesis of silver nanoparticles (AgNPs) from ethanolic leaves extract of *Volkameria inermis*

Silver nanoparticles were prepared from ethanolic leaves extract of *Volkameria inermis*. To 10ml of the ethanolic leaves extract 90 ml of 1mM silver nitrate solution was added and exposed to bright sunlight for 20 minutes[11].

Functionalization of AgNPs with PEG

Weighed 2mg of polyethylene glycol and dissolved in 20ml of distilled water which was heated at 90ºC. To 50ml of synthesized AgNP solution added 3ml PEG stock solution and stirred continuously for 2 hours in a magnetic stirrer[12].

Analysis of Biocompatibility Role

Hemolytic Assay

Ethylene diamine tetra acetic acid (EDTA)-stabilized human blood samples were freshly

collected and used within 3 hours of being drawn. 4ml of whole blood sample was added to 8ml of phosphate-buffered saline (PBS). The PBS solution was formulated to the following composition (mM): 0.14NaCl, 2.7KCl, $10Na_2HPO_4$ and $1.8KH_2PO_4$, and the pH was adjusted to 7.4. The RBCs was isolated from blood by centrifugation at 10000rpm for 5 min. The RBCs were further washed five times with sterile PBS solution. Following the last wash, the RBCs were diluted to 40ml of PBS. Then 0.2 ml of diluted RBC suspension was added to PEGylated AgNPs solution at systematically varied concentrations and mixed by vortexing. All the sample tubes were kept in static condition at room temperature for 3 hours. Finally, the mixtures was centrifuged at 10016g for 3 min and 100ml of supernatant of all samples was taken and its absorbance was recorded on a spectrophotometer (Systronics UV-vis Spectrophotometer) at 545nm. The percentage hemolysis was calculated using the following relationship,

$$\text{Hemolysis \%} = \frac{\text{Sample absorbance} - \text{Negative control}}{\text{Positive control} - \text{Negative control}} \times 100$$

In which, RBC incubation with distilled water and PBS were used as the positive and negative controls, respectively[13].

***In vitro* Drug Releasing Profile**

The drug release response from polymer modified nanoparticles was investigated at the physiological temperature of 37ºC and pH of 7.4, 6.8 and 5.5. The medium of pH7.4 corresponds to the physiological pH while pH 6.8 simulates the pH of tumor tissue and pH of 5.5 corresponds to the mature endosomes of tumor cells. The release profiles of PEGylated AgNPs were studied using a dialysis bag (MWCO-3500). PEGylated AgNPs was dispersed in 10ml of the respective PBS buffer allowed to stabilize for 30 min and then placed in a dialysis bag. The dialysis bag was immersed in 50ml of PBS solution (pH 7.4, 6.8 and 5.5) in a beaker and then placed at 37ºC. At predetermined time intervals 3ml of the release medium was collected to measure the released drug concentration and then was replaced with the same fresh PBS. For the measurement of released AgNPs concentration, the absorbance of the release medium at 480nm was recorded on a Systronics UV-vis absorption spectrophotometer. Experiments for all samples were performed three times at each pH value[14].

RESULTS AND DISCUSSION

Effect of PEGylated AgNPS on the Extent of Hemolysis

The toxicity study of PEGylated AgNPs synthesized from the leaves of *Volkameria inermis* was tested on human red blood cells from healthy volunteers to determine the biocompatibility. Hemoglobin release analysis (Table No.3) showed the hemolytic control and PEGylated AgNPs at different concentrations. When water was added to RBC's hemolysis takes place and the released haemoglobin was measured. This serves as the positive control and represents 100% hemolysis. RBCs suspended with PBS showed 0% hemolysis, which was taken as the negative control.

Effect of PEGylated AgNPs on the Morphology of Human Blood Cells

The non-toxicity of the synthesized PEGylated AgNPs was further investigated based on the changes in the morphology of human red blood cells incubated with PEGylated AgNPs. The morphology of the red blood cells was observed using an inverted microscope and the photographs are shown Figure No.3.

The cell morphology analysis indicated that incubation of RBCs with 200µg/ml of PEGylated AgNPs did not result in hemolysis or change in morphology of red blood cells when compared with that of the control. Thus, the cell morphology analysis was corroborated with that of haemoglobin release analysis signifying the biocompatible nature of PEGylated AgNPs.

In our study, the absence of hemolysis was due to the surface modification of AgNPs by the biocompatible polymer PEG which prevented the adhesion of both the nanoparticles and the red blood cells. Thus, this simple surface modification stratagem ensures the safety of PEGylated AgNPs for biomedical applications.

The PEGylated AgNPs synthesized from the leaves of *Volkameria inermis* did not exhibit any toxicity to the red blood cells in the *in vitro* toxicity tests (hemolysis and cell morphology) studied. These observations clearly show that the PEGylated AgNPs are biocompatible for the human system.

Drug Release Profiles of PEGylated AgNPs

The toxicity study was followed by the drug release profiles of PEGylated AgNPs prepared from the leaf extract of *Volkameria inermis* selected for analysis was recorded from 0 hour up to 48 hours. The PEGylatedAgNPs showed an obvious pH related release behaviour. The profile showed that at pH 7.4 the release rate was slow and sustained with a release per cent of 36 ± 0.23% in 48 h. At pH 6.8 the drug release was higher than that at pH 7.4. The release per cent was found to be 61±0.29 in 48h. Whereas, at pH 5.5 the drug release was more rapid with approximately 81±0.39 within the same period indicating the sensitivity of the drug towards endosomal pH.

At pH 7.4 the drug release was slow and sustained, this is attributed due to the hydrazide linkage of PEGylated AgNPs remained stable for a considerable period of time during circulation in blood and thereby eliminates a premature burst release. Such stability, for a prolonged period of time can reduce the side effects of the drug on normal cells. At pH 6.8 the drug release was higher than at pH 7.4, probably due to the slight protonation effect of the hydrazide linkage. However, at pH 5.5 a drastic release was obtained, since at lower pH degradation of hydrazide bond occurred which enabled a drastic release of phytocomponents. Thus this study showed the drug ability of PEGylatedAgNPs towards the tested pH conditions.

Thus, at all the pH conditions the drug release steadily increased upto 16 hours. After which, a steady plateau was obtained indicating the sustained release of the drug which improves the therapeutic efficacy with low side effects.

The results obtained, concluded that PEGylated AgNPs synthesized from *Volkameria inermis* extract showed a steady release of phytocompounds, indicating that this PEGylated AgNPs have a strong potential to contribute as a drug delivery system.

Discussion

The production of silver nanoparticles (AgNPs) using chemical synthesis routes require hazardous and toxic solvents. Nowa-days, there is an increasing requirement to develop safe and eco-friendly methods, which do not apply harmful chemicals in the synthesis routes.

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The genus *Volkameria* has been reported that it has many medicinal properties which is used to treat antioxidant, anti inflammatory and anticancer which was reported[15]. Flavanoids was extracted from *Volkameria inermis* by using different solvent extractions and ethyl acetate solvent fraction was showed better cytotoxic effect against ELA induced mice model was reported[16]. Based on the above evidence our results are more supportive for the drug designing, when PEGylated AgNPs was added the percentage of hemolysis was found to be less than 5% comparable to the negative control. It has been reported that upto 5% hemolysis was permissible for biomaterials[17]. The largest percentage of hemolysis at the tested concentration was found to be 1.24 ± 0.04% for 200µg/ml. Since this is much lower than 5% PEGylated AgNPs was considered as hemocompatible for drug delivery applications.

Similarly, several other studies have reported that lower toxicities were observed for biologically synthesized silver nanoparticles than the chemically synthesized ones. PEG coated on the surface of

mesoporous silica nanoparticles did not result in apparent hemolysis after 3h blood incubation[18]. Similarly, the alcoholic extract of tulsi leaf mediated silver nanoparticles did not show any red blood cell lysis when compared with conventional drug[19]. In another study, the silver nanoparticles synthesized from aloe vera exhibited low toxicity[20].

Silver nanoparticles synthesized from the leaf of *Olaxscandens* showed a high release of silver ions in acidic environment under *in vitro* conditions[21]. Similarly, in another study the *in vitro* release of microanozle from microanozle- AgNPs was found to be higher at acidic pH[22].

Figure No.1: *Volkameria inermis*

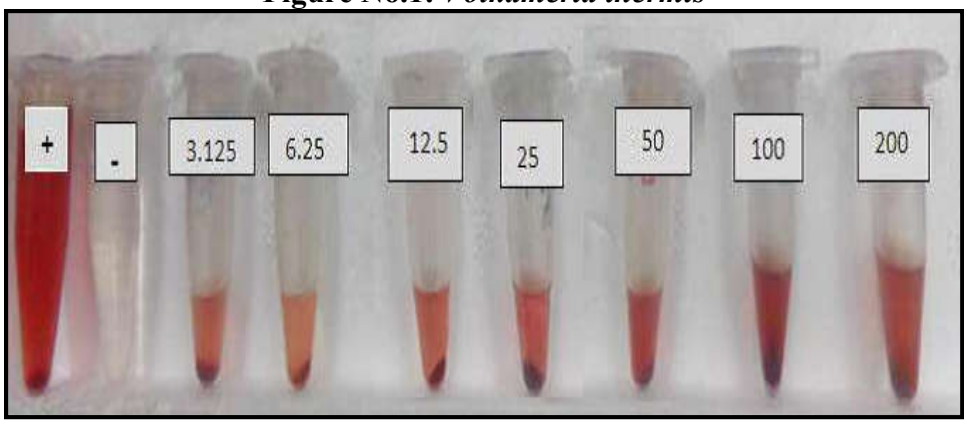

Figure No.2: Percentage of hemolysis at different concentration

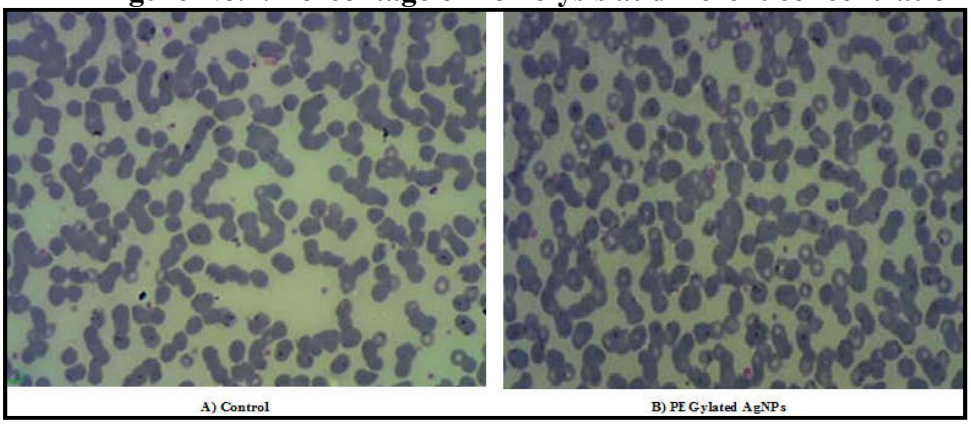

Figure No.3: Microscopic image (Magnification at 40X) of human RBC treated with PEGylated AgNPs (200µg/ml)

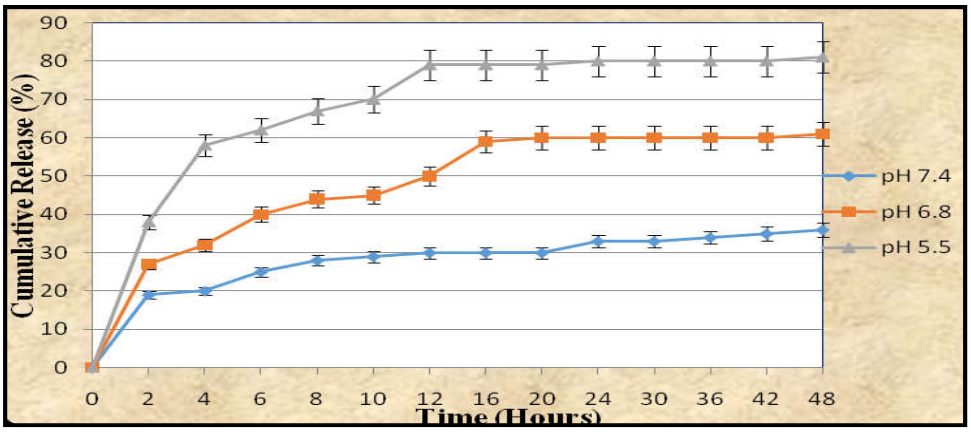

Figure No.4: Drug release profile PEGylated AgNPs synthesized from the leaves of *Volkameria inermis*

Table No.1: Percentage of hemolysis at different concentrations of PEGylated AgNPs

S.No	Concentration of PEGylated AgNPs (µg /ml)	Percent Hemolysis
1	Positive Control	100 ± 0.01
2	Negative Control	0.001 ± 0.00
3	3.125	0.3 ± 0.01
4	6.25	0.4 ± 0.03
5	12.50	0.5 ± 0.1
6	25	0.5 ± 0.09
7	50	0.6 ± 0.01
8	100	0.7 ± 0.02
9	200	1.24 ± 0.04

CONCLUSION

In Our research work an eco-friendly green synthesis method was adopted for the synthesis of silver nanoparticles. The synthesized silver nanoparticles were functionalized using PEG. The functionalized silver nano was studied for their biocompatible and drug releasing profile at different pH conditions. PEG coated silver nano was highly biocompatible and showed a good drug releasing profile towards endosomal pH.

We believe that these findings are basic steps which are important for understanding interactions between AgNPs and human platelets and their pharmacological and toxicological effect. Further studies are warranted for establishing the safety profile of these AgNPs prior to their potential clinical applications.

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DECLARATIONS

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CONFLICT OF INTEREST

The authors report no conflicts of interest in this work.

ETHICAL APPROVAL

This work was property done with Ethical committee members of Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore.

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