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Development and Characterization of Gold Nanoparticles Conjugates to Increase Bioavailability of 6-Gingerol

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

6-Gingerol, an abundant component of *Zingiber officinale,* acts as a cardiotonic and is also used in the treatment of cancer disease, but its low solubility makes it very challenging in therapeutic applications. As we are all aware of the metal toxicity of nanoparticles, here we are using gold metal because gold nanoparticles are found to have lower toxicity than other metals. In this study, we prepared optimized conjugated gold nanoparticles of 6-Gingerol (Au-6G-PVP-NPs) by chemical reduction method using polyvinylpyrrolidone, a biocompatible and biodegradable polymer, to increase the bioavailability and solubility of 6-Gingerol. The prepared nanoparticle conjugate was evaluated on different parameters such as pH, solubility, Zeta potential, TEM, DLS, polydispersity index, in vitro release, and stability studies to meet the criteria. The study concluded that gold nanoparticles conjugated with 6-Gingerol showed good solubility in gastric pH, effective drug

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release, and were more stable than free 6-Gingerol. Hence, it can be concluded that these conjugates can be used for cardiac disease as well as cancer treatment due to their good bioavailability, drug release, improved biocompatibility, stability, and decreased cytotoxicity.

Keywords: 6-Gingerol; gold nanoparticle; polyvinylpyrrolidone; Au-6G-PVP-NPs.

1. INTRODUCTION

Nanoparticles offer several advantages and disadvantages in biomedical applications. On the positive side, nanoparticles have unique properties such as a larger surface area, better targeting capabilities, and the ability to penetrate tissues, making them promising materials for drug delivery and cancer treatment. They also exhibit size-dependent characteristics and improved particle size distribution and shape, which can enhance their performance in various applications. Additionally, nanoparticles can be used for disease diagnostics, medical imaging, and tissue engineering. However, there are also potential drawbacks to using nanoparticles. Some nanomaterials, such as carbon nanotubes and metallic nanoparticles, have been found to be toxic to cells and can induce oxidative stress and genotoxicity. Furthermore, the development of resistance to nanoparticles as antimicrobials is a concern. It is important to carefully consider the safety and potential side effects of nanoparticles in biomedical applications [1].

The application of metal nanoparticles has drawn much attention, offering significant advances, especially in the field of medicine, by increasing the therapeutic index of drugs through site specificity, preventing multidrug resistance, and delivering therapeutic agents efficiently. The use of metallic nanoparticles for drug delivery systems has significant advantages, such as increased stability, solubility, bioavailability, and half-life of the drug carrier in circulation, required biodistribution, and passive or active targeting into the required target site. The advantages and disadvantages of different metallic nanoparticles like silver, gold, iron, copper, zinc oxide, and titanium dioxide in drug delivery systems are given in Table 1.

Table 1. Illustration of types of nanoparticles, their advantages & disadvantages

Nanoparticle Class	Advantages	Disadvantages
Gold Nanoparticles (AuNPs)	Strong Biocompatibility	Chemical contaminants from synthesis can cause toxicity issues
	Established delivery platform for a variety of cancer drugs	Less direct anti-cancer effects than other nanoparticle materials
Silver Nanoparticles (AgNPs)	Good biocompatibility	Size-dependent cytotoxicity requires tuning of particle size
	Direct anti-cancer cell killing capability	Potential off-target effects with little delivery to the tumor
Iron Oxide Nanoparticles (IONPs)	Ability to direct uptake through external magnetic stimulation	Acting targeting requires significant research to achieve clinical utility
	Can be functionalized with ligands to enhance active targeting	
Zinc Oxide Nanoparticles (ZnONPs)	Innate action on molecular pathways inducing ROS, cytokine and chemokine secretion, and cancer cell apoptosis	Off-target effects with poor tumor accumulation must still be addressed in vivo
Titanium Dioxide Nanoparticles (TiO_2NPs)	Similar direct cytotoxicity mechanisms as ZnONPs, through ROS generation and DNA damage to cancer cells	NPs frequently accumulate in RES organs are cleared through the renal system before significant tumor accumulation

Gold nanoparticles (AuNPs) have been widely employed in bio-nanotechnology based on their unique properties and multiple surface functionalities. The ease of AuNP functionalization provides a versatile platform for nano-biological assemblies with oligonucleotides, antibodies, and proteins [2]. Bioconjugates of AuNPs have also become promising candidates in the design of novel biomaterials for the investigation of biological systems [3].

The versatility of AuNPs has provided useful materials for a range of biomedical applications. In diagnostics, the binding event between the analytes and the AuNPs can alter the physicochemical properties of AuNPs, such as surface plasmon resonance, conductivity, and redox behaviour, leading to detectable signals[4]. AuNPs also serve as practical platforms for therapeutic agents, with their high surface area allowing a dense presentation of multifunctional moieties (e.g., drugs and targeting agents).

Gold nanoparticles (AuNPs) have recently emerged as a potential delivery system that efficiently transports and distributes pharmaceutical products to various types of cells. It has also numerous other applications in the domains of biomedical imaging and diagnostics for the detection of different illnesses, owing to its unique physical and chemical characteristics compared with either small molecules or other bulk materials. AuNPs can be synthesized via chemical reduction, transforming metallic gold into nano-particular gold. Different chemical methods for stable and large-scale AuNP production, were described by Turkevich in[5], like citrate mediated reduction. However, the most often used chemical technique of generating AuNPs was seed media growth reported in 1996. As a result, several synthesis techniques of different gold nanostructures were successfully employed, however their toxicity restricts its potential for use. Eco-friendly or green chemistry and non-toxic biological or biomimetic techniques were therefore also frequently used for AuNP production [6]. One important problem in potential therapy is that toxicity must be carefully examined. Even though it has been reported that gold nanoparticles are inherently non-toxic, it is important to discern the toxicity of the nanoparticle core and that of its capping ligands. Some toxicity may be specific to certain ligands. For example, cationic ligands clearly cause moderate toxicity *in vitro* [7]. Equally important is considering how the conjugated ligands may change the pharmacokinetics, biodistribution, and eventual side effects.

Worldwide, people use the rhizome of Zingiber officinale (ginger) as a spice and herbal remedy. It includes strong phenolic compounds referred to as gingerols. The main pharmacologically active ingredient in ginger is gingerol. 6-Gingerol, the aromatic compound that was separated from ginger oleoresin, has been shown to have strong antioxidant properties based on its ability to suppress phospholipid peroxidation, which is triggered by the $FeCl_3$-ascorbate system. Inflammation caused by phorbol ester is inhibited by gingerol. The biological activity, including cancer, anti-inflammation and anti-oxidation, is known to be varied [8]. The effect of 6-Gingerol on several biological pathways including apoptosis, cell cycle regulation, cytotoxic activity and Angiogenesis inhibition was found to have anticancer properties [9]. Thus 6-gingerol has received a significant interest as a potential therapeutic agent for the prevention and/or treatment of many diseases because of its effectiveness and regulation of multiple goals, as well as its safety for human use [10].

In this study we prepared polymeric PVP- 6-gingerol conjugated and Metallic *cum* Polymeric AuNP-PVP-6-gingerol conjugated nanoparticles. The formulations developed thus overcome the drawback and limitation of conventional drug delivery systems and subsides critical solubility issues. Study concluded of improved bioavailability of gingerol, which will maximizing the drug action on desirable site, decrease the metabolism of drug before reaching to desirable site of action, Decrease the body-wide distribution of drugs to help maintain the required concentration at the desired site [11].

2. MATERIALS AND METHADOLOGY

2.1 Formulation of gold Nanoparticles (AuNPs)

Citrate capped AuNPs synthesized by adding 95 ml. of chloroauric acid solution (containing 5 mg. Au) were heated to the boiling point and 5 ml. of 1% tri-sodium citrate ($Na_3C_6H_5O_7$) used as a metallic nanoparticle precursor and reducing agent respectively. 100ml of 1mM $HAuCl_4$ was boiled under reflux condenser with continue stirring for 20 min. 10ml of 38.8mM tri sodium citrate was added directly into the boiled solution. Color of the solution has been changed from pale

yellow to colorless and finally dark reddish within 5 minutes. [12]

Table 2. Formulation plan for gold nanoparticles

Ingredients	Concentration
Chloroauric acid (HAuCl4)	1mM
Sodium citrate (Na3C6H5O7)	38.8mM

2.2 Preparation of Master Formula: Conjugation of 6-gingerol with PVP

Tri-sodium citrate and 6-gingerol both act as capping and stabilising agents. 100 ml of 3.88 mM tri-sodium citrate in water heated under a reflux condenser and 10 mg of polyvinyl pyrrolidone dissolved in 20 ml of water were added to 100 ml of tri-sodium citrate solution and boiled for 45 min. The solution was cooled, and for the conjugation of 6-gingerol dissolved in 25 ml of methanol, the solution was added to the above solution, and the mixture of reactions that was stirred continued for 3 hours at 60^0C for the full removal of methanol from the solution with no reflux condenser. The PVP-6-gingerol conjugated solution was then centrifuged at 12000 rpm for 40 min at 4.0 ºC to remove the unattached 6-gingerol. This centrifugation has been done three times to ensure that no free 6-gingerol molecules are left in the final conjugate. The final solution was used for a spectroscopic study and TEM analysis. Part of this concentrated solution will also be used for release rate analysis [13].

Table 3. Formulation plan for PVP-6-gingerol NPs

Ingredients	Concentration
6-Gingerol	40mg
Sodium citrate (Na3C6H5O7)	38.8mM
Polyvinyl Pyrrolidone	10mg

2.3 Preparation of Master Formula: Conjugation of 6-Gingerol with PVP and AuNPs

Polyvinylpyrrolidone is used as a release retardant material. Prepared citrate-capped gold nanoparticles were heated for 10 minutes before adding a PVP solution (10 mg PVP in 20 ml water) to the AuNPs solution and stirring for an additional 45 minutes. For the conjugation of 6-gingerol, 40mg of 6-gingerol is dissolved in 25 ml of methanol, and the solution is added to the above solution. The mixture of reactions that was stirred continues for 2 hours at 60 oC for the full removal of methanol from the solution with no reflux condenser.

AuNP PVP-gingerol conjugated solution was then centrifuged at 12000 rpm for 40 min at 4.0 ºC to remove the unattached 6-gingerol. This process has been done three times to ensure that no free 6-gingerol molecules are left in the final conjugate. The final solution was used for a spectroscopic study and TEM analysis. Part of this concentrated solution is also used for release rate analysis.

Table 4. Formulation plan for AuNPs-PVP-6-gingerol nanoparticles

Ingredients	Concentration
6-Gingerol	40mg
Chloroauric acid (HAuCl4)	1mM
Sodium citrate (Na3C6H5O7)	38.8mM
Polyvinyl Pyrrolidone	10mg

2.4 Characterization of Nanoparticles [14-18]

The formulated nanoparticles were evaluated for chemical interaction, drug content uniformity, particle size and shape, drug loading, entrapment efficacy, surface charge, in-*vitro* drug release study, and stability. The prepared PVP-6-gingerol conjugates, gold nanoparticles, and Au-PVP-6-gingerol conjugates samples were characterised by UV-Vis spectroscopy (Shimadzu Corporation, Japan), Fourier transform infrared spectroscopy (FTIR-Perkin Elmer), high-resolution transmission electron microscopy (HR-TEM,JEOL Ltd., Japan), The drug release rate has been done in various pH conditions, with the surface charge of nanoparticles characterised by the zeta sizer (Malvern), simultaneously PDI of nanoparticles and drug loading calculated.

2.5 Chemical Interaction and Conjugation

Conjugation of 6-gingerol with PVP as well as AuNPs confirmed by the Scanning graph of UV spectrophotometer and chemical interaction confirmed by FTIR.

a) UV-Vis spectroscopy

For the UV spectroscopy, methanol used as a solvent, baseline always has done before

scanning any sample, Surface Plasmon Resonance (SPR) of gold nanoparticle also evaluated by UV absorbance. Most of the time 200µl diluted up to 1ml concentration was used for UV analysis.

b) Spectrum analysis for compound [19]

The absorption of a 6-gingerol using 80% methanol was monitored by measuring its absorption spectrum with the help of a UV-Vis spectrophotometer (UV1800, Shimadzu). The operational range of wavelength was varied from 260nm to 300nm. The absorption spectrum of the extracted sample was obtained in graphical form. The ideal peak should be obtained at 279-280nm [20]. The peak absorbance was obtained at 279nm which indicates that 6- gingerol was indeed present in the sample.

c) FTIR Spectroscopy

Infrared spectrum of 6-gingerol, PVP and conjugated NPs were determined by using FTIR Spectrophotometer using KBr disks method for 6-gingerol and PVP and nujol mull method for conjugated NPs. Mortar and pestle may be used for grinding and mixing. The base line correction was made with potassium bromide and nujol dried. The spectrum of dried sample mixtures and bromide was then scanned using the reference graph from 4000 cm^{-1} to 400 $cm^{-1.}$

d) Morphology (Shape and Size)

The morphology and size of nanoparticles were determined by Transmission electron microscopy (HR-TEM) and Dynamic light scattering technique.

e) Surface charge

The zeta potential and surface charge of nanoparticles were determined by the Zeta Potential Analyzers. The zeta potential of a nanoparticle is commonly used to characterize the surface charge property of nanoparticles.

f) Percentage Yield

The efficiency of any method is calculated and helps in selecting a suitable fabrication technique. The practical yield was determined in relation to the total of the starting material by weight of nanoparticles recovered from the batch. The following formula may be used to calculate:

$$PY(\%)=(Nanoparticlesweight/Theoreticalmass) \times 100$$

g) Polydispersity Index (PDI)

Polydispersity gives the measurement of aggregation and agglomeration of nanoparticle. PDI or heterogeneity index, is a measure of the distribution of molecular mass in a given polymer sample. The PDI calculated is the weight average molecular weight divided by the number average molecular weight i.e. PDI = Mw/Mn.

h) Drug Loading

Centrifugation method was used to determine the content of drugs. A centrifugal suspension of the nanoparticles was made at 12000 rpm at 4.0°C for 20 min to separate the free drug into the supernatant. Using a UV-visible spectrophotometer at 281 nm after suitable methanol dilution, the concentration of 6-gingerol in the supernatant was measured. The loading of drugs (DL %) was determined using equation.

$$DL\ \% = \frac{A\ (total\ \ drug\ wt) - A''(free\ drug\ wt)}{A(total\ drug\ wt)} \times 100$$

i) *In- vitro* drug release

A dissolution media, PBS buffer and phosphate buffer are employed. The experiment was conducted at 35±0.5°C in the pH 4, 5, 6 (phosphate buffer) and 8 (PBS) buffer. PBS buffer is working better above pH6 therefore phosphate buffer has taken for lower pH. Literature stated that greatest stability observed at low pH. A known amount of NPs dispersed in a 30ml buffer medium and a specified sample volume was drawn out at each time interval and concurrently replaced with the same fresh medium volume, and UV vis spectroscopy study was performed in the release kinetics. Data have been recorded for release control with specific time intervals.

Table 5. Parameters used during *in-vitro* release study

S.No.	Parameter	Range
1	Buffer media	Phosphate buffer and PBS buffer
2	Temperature	37±0.5°C
3	Speed	50rpm
4	Time intervals	0.5, 1.0, 2.0, 12hr

3. RESULTS AND DISCUSSION

Critical solubility and bioavailability issues of 6-gingerol has been resolved by formulating its nanoparticle conjugates with gold nanoparticles (AuNPs) and polyvinyl pyrrolidone (PVP) and each conjugation step subjected to evaluation parameters like particle morphology, chemical interaction, drug loading and entrapment efficiency, Drug content uniformity and *In-Vitro* drug release study.

3.1 Characterization of AuNPs-PVP-6-Gingerol NPs

3.1.1 UV-Vis spectrum of conjugated nanoparticles

UV-vis absorption spectra of the samples at different reaction times were taken to further investigate. UV spectra provide the evidence of conjugation between AuNP-PVP-6-gingerol. In Fig. 1 of UV spectra of a) 6-gingerol in methanol; b) PVP-conjugated 6-gingerol; c) citrate-capped AuNPs; d) 6-gingerol-conjugated PVP-AuNPs. Surface plasmon resonance peaks of pure 6-gingerol with methanol were found at 279 nm, and the PVP-conjugated 6-gingerol peak was shifted to 287 nm due to the increase in size after PVP conjugation with 6-gingerol. Another SPR absorption peak of citrate-capped AuNPs shows at 510 nm; after conjugation, the 6-gingerol-conjugated PVP-capped AUNPs peak shifted to 529 nm due to the increase in size after PVP capping at the initial stage of the reaction and maintained its position after the formation of the gold nanoparticles, which implied that capping agents effectively prevented any possible further growth or agglomeration of the as-prepared nanoparticles.

3.1.2 FTIR spectrum analysis [21]

From the UV-Vis spectroscopic evidence of 6-Gingerol conjugation, here also FTIR analysis has done for conjugated nanoparticles and compared it with free 6-Gingerol and PVP. In Fig. 2 FTIR spectrum of 6-Gingerol, PVP and conjugated AuNP-PVP -6-Gingerol clearly shown. Functional group which are shown in FTIR spectrum and characteristic peaks described in Table 5.Peak corresponding to $–CH_2$ stretching are clearly appearing at 2945 cm^{-1} .In conjugated NPs spectra except the free OH- group of 6-Gingerol at 3512$cm^{-1}$and C=O group of PVP at 1656 cm^{-1} remain same but these two functional group peak shifted to right side 3436 cm^{-1} for 6-Gingerol and 1636 cm^{-1} formation of a hydrogen bonding between OH-group of 6-Gingerol and C=O group of PVP and both functional group responsible for binding.

3.2 Morphological Characterization

3.2.1 HR- Transmission electron microscopy

The synthesized AuNPs were characterized for HR-TEM. The size of simple gold nanoparticles were around 10 nm-50 nm have shown in Fig. 3(a) & (b). Prepared PVP-6-gingerol conjugates were spherical in shape and the contrast of the nanoparticle is different in inner and outer core due to the deposition of gingerol to the outer surface of PVP nanosphere. Most of the NPs size is 200 nm (Fig. 4 a & b), but some are larger and smaller from this size range.

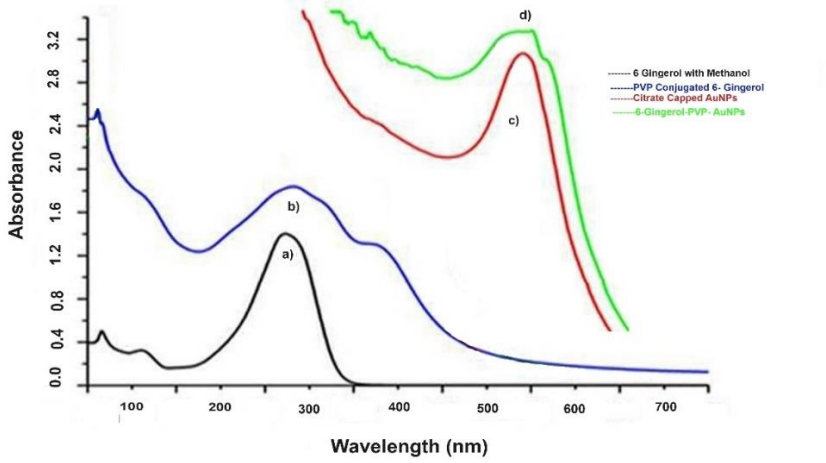

Fig. 1. UV-Vis Spectrum of Au-PVP-6-Gingerol conjugates in various stages

Table 6. Characteristic Peak of 6-Gingerol with PVP Capped

S.No	Name	Reference peak(cm-1)	Obtained peak(cm-1)	Functional group
1.	Gingerol	3550-3500	3512.23	Free OH- stretching
2.	Gingerol	2840-2950	2945.30	C-H Methyl
3.	Gingerol	3015	3015.88	C-H Aryl
4.	Gingerol	1450-1630	1461	C=C Aromatic
5.	PVP	1660	1656	C=0
6.	PVP	1230	1229	C-N

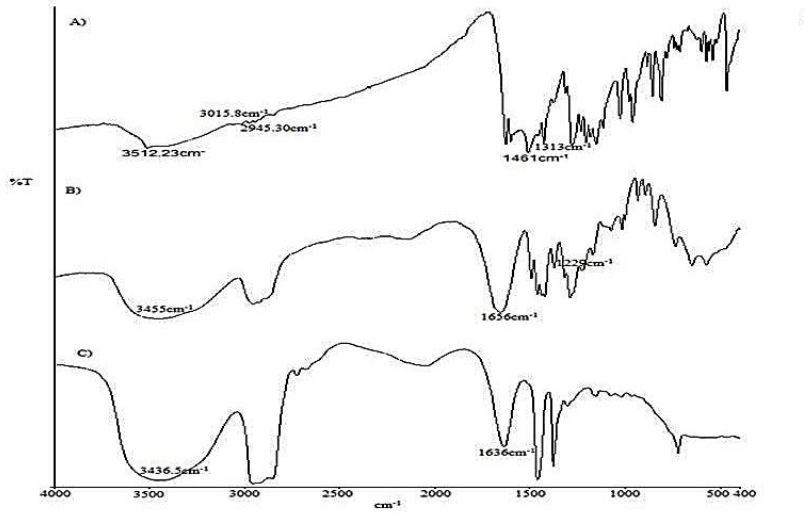

Fig. 2. FTIR spectrum of a) 6-Gingerol, b) PVP capped AuNPs, c) Conjugated AuNPs-PVP-6-Gingero

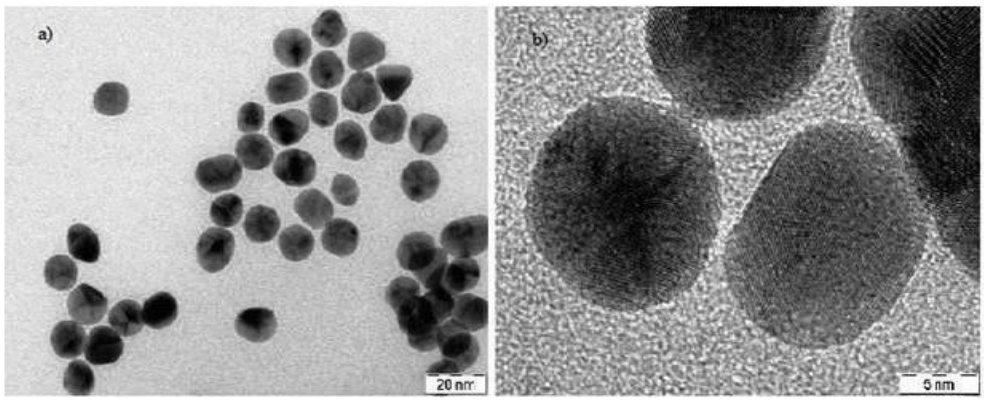

Fig. 3. a) HR-TEM images of prepared AuNPs, b) Magnify images

Prepared AuNPs-PVP-6-Gingerol conjugated nanoparticles were spherical in shape are shown in Fig. 5 - HR-TEM of a) citrate capped AuNPs, b) PVP conjugated AuNPs, c) 6-Gingerol conjugated PVP-AuNPs. From the HR-TEM result it has been found that AuNPs is 10 nm-50 nm size range and contrast of the inner core and outer is same but after PVP capping size slightly increase with different contrast in outer and inner part, after addition of 6-Gingerol, free PVP form a large nanosphere around 200nm and PVP conjugated AuNPs and 6-Gingerol both attached to the surface of PVP nanosphere. Therefore, in final conjugation (AuNPs-PVP-6-Gingerol.) most of the NPs size are around 200 nm (Fig. 5 a, b, c,& d).

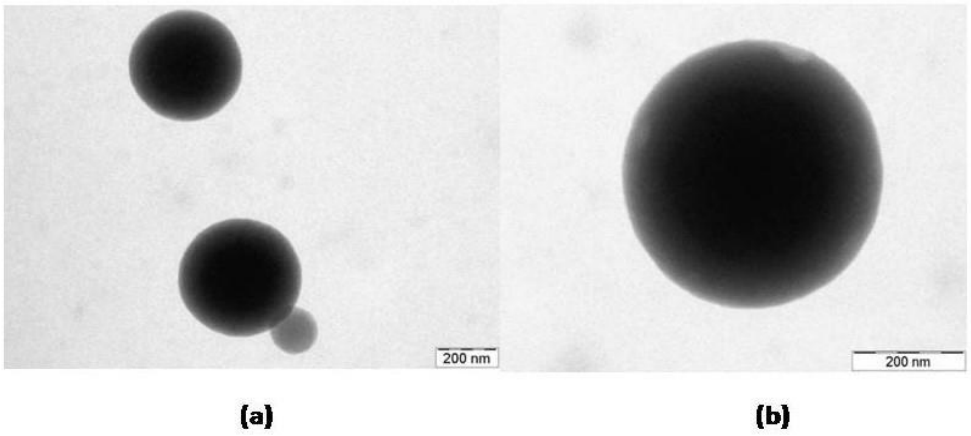

Fig. 4. a) HR-TEM images of PVP-conjugated NPs, b) Magnify images of PVP-conjugated NPs

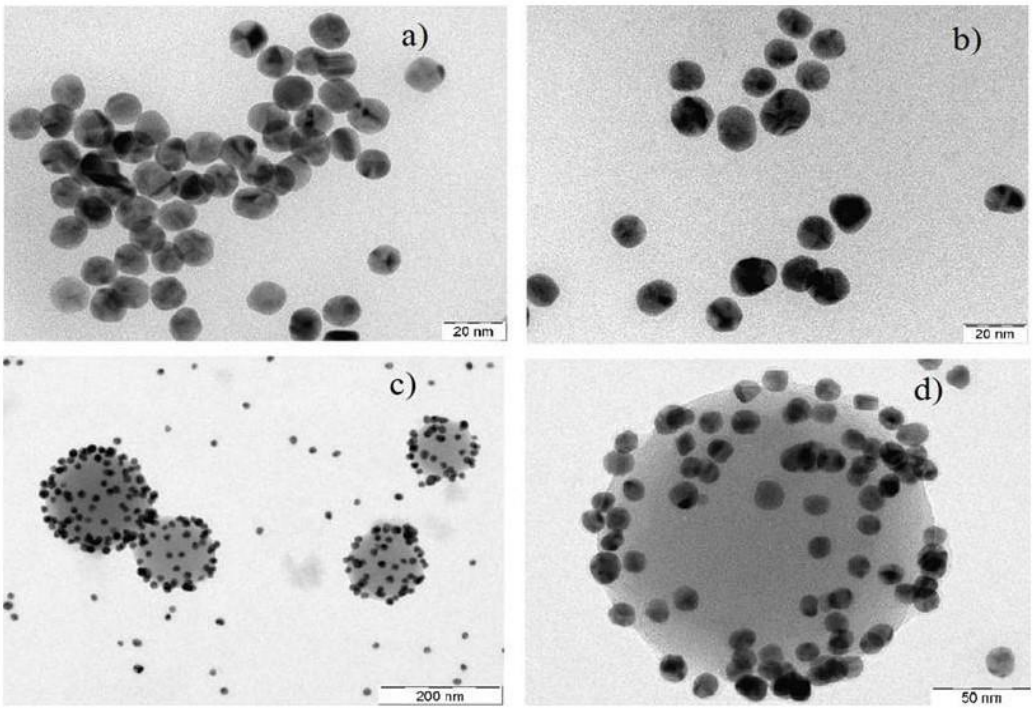

Fig. 5. HR-TEM images of a) AuNPs, b) PVP capped AuNPs, c) 6-Gingerol-PVP-AuNPs d) Magnify images of conjugated NPs

3.2.2 Dynamic Light Scattering (DLS) And Zeta Potential

The AuNPs were also characterized for dynamic light scattering (DLS) and zeta potential. (Fig. 6 (a) & (b) The hydrodynamic size of AuNPs was found to be around 39.26 nm which is comparable with TEM size. The zeta potential gives charge on nanoparticle surface which was found to be -4.06 mV. The value of zeta potential tends to under the range of strong agglomeration and precipitation. Which may be shows the solubility issue of nanoparticle.

Further size of NPs confirmed by the DLS study (Fig. 6 a & b) and average size range of nanoparticle obtained 220.5 nm. For zeta potential measurement water was used as a solvent for dispersing the nanoparticles. The zeta potential of the PVP-6-gingerol nanoparticles was -36.2 mV. The high surface charge causes the greater stabilization of the nanoparticles.

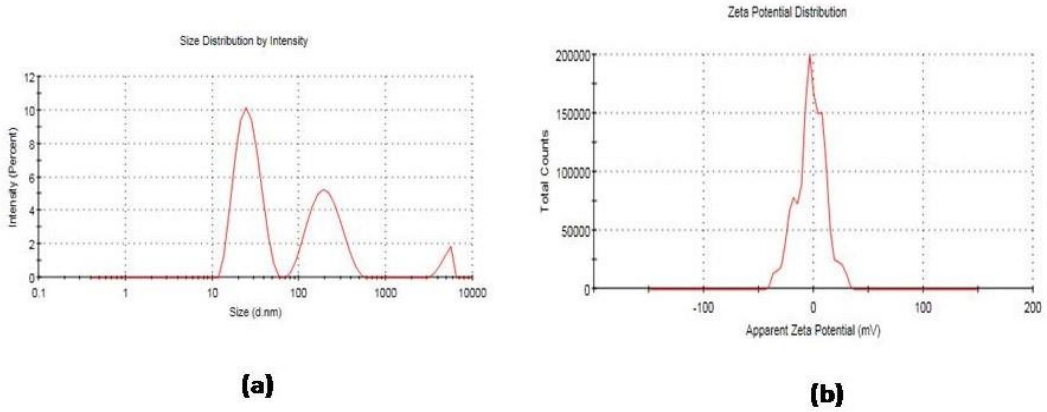

Fig. 6. a) Zeta Potential and surface charge b) Average size distribution of AuNPs

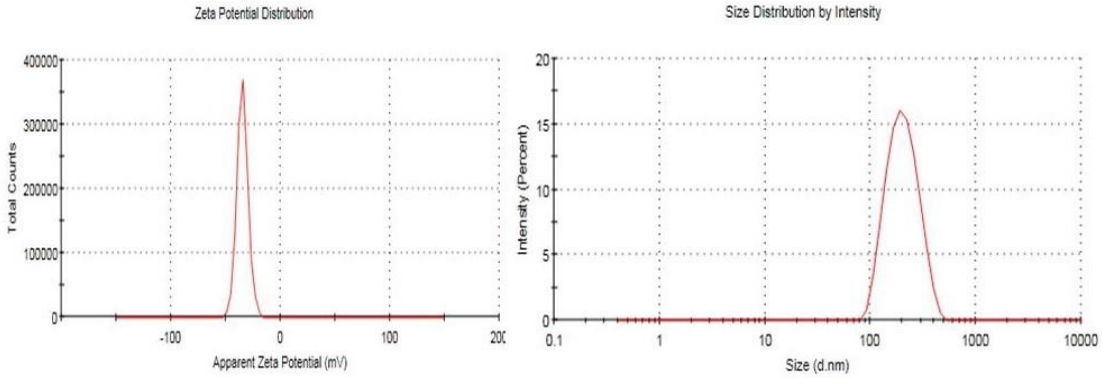

Fig. 7. a) Zeta Potential and surface charge b) Average size distribution of PVP conjugated AuNPs

The zeta potential of the AuNPs-PVP-6-Gingerol nanoparticles was -50.1 mV. Further size of NPs confirmed by the DLS study and average size range of citrate capped AuNPs was 39.26 nm, after PVP capping size was increased to 220.5 nm and final conjugate size was 251.8 nm obtained. (Fig. 8 a & b) and Table no 7. For zeta potential measurement water used as a solvent for dispersing the nanoparticle. The zeta potential of the AuNPs was found to be -4.06 mV, and after PVP conjugation charge increased to -36.40 mV and AuNPs-PVP-6-Gingerol conjugates have shown -50.1 mV. (Fig. 8) the high surface charge of final conjugates causes the greater stabilization of the nanoparticles.

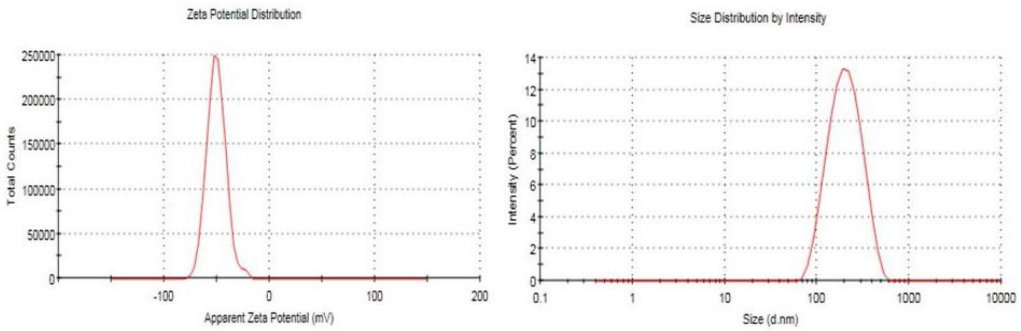

Fig. 8. a) Zeta potential or surface charge and, b) average distribution spectra of AuNPs-6-Gingerol- PVP Conjugates

Table 7. Zeta potential and size distribution study of AuNPs, PVP-6-Gingerol Conjugate &AuNPs-6-Gingerol-PVP

S.No.	Sample Type	Zeta Potential Value	DLS Value
1	AuNPs	-4.06 mv	39.26nm
2	PVP-6-Gingerol Conjugate	-36.40mV	220.5 nm
3	AuNPs-6-Gingerol-PVP conjugates	-50.1mV	251.8nm

3.2.3 Drug loading and polydispersity index (PDI) of nanoparticles

a) Drug loading was determined by centrifugation method. The redispersed nanoparticles were centrifuged at 12000 x g for 30 min at 4.0°C to separate the free6-Gingerol. Concentration of 6-Gingerol in the supernatant was determined by using UV-Vis spectrophotometer at 221 nm. The drug loading (DL%) was determined using this equation.

$$DL\ \% = \frac{A\ (total\ \ drug\ wt) - \ A''(free\ drug\ wt)}{A(total\ drug\ wt)} \times 100$$

$$DL\% = (40mg - 11.43mg\ /40mg) \times 100$$

DL%= 71.42

b) Polydispersity Index (PDI)

Polydispersity gives the measurement of aggregation and agglomeration of nanoparticle. PDI value of AuNPs-PVP-6-Gingerol NPs from DLS measurement was 0.622 for AuNPs, 0.474 for PVP capped AuNPs and 0.191 for final conjugate AuNP-PVP-6-Gingerol. Less PDI value of final conjugate concluded that conjugated NPs were mono dispersed which facilitates its stability characteristics for long time.

3.3 Stability Studies b/w free 6-Gingerol and conjugated Nanoparticles

It has already shown in Fig. 9 the degradation of free 6-Gingerol in aqueous medium, but after conjugation, the degradation rate of 6-Gingerol shown in Fig. 9, in this stability study only 17% 6-Gingerol was degraded within 21 days. Therefore, it can be concluded that AuNPs-PVP binding with 6-Gingerol provided the greater stability to the 6-Gingerol. Conjugated NPs also stable in aqueous medium for long time.

3.4 In-Vitro Drug release studies

The experiment was conducted in pH 4, 5, 6, (phosphate buffer), and 8 (PBS) buffer at 35±0.5°C at gentle agitation, as measured by the in-vitro release study with a direct dispersion method (PBS) and a phosphate buffer. The *in-vitro* drug release from AuNPs-PVP-6-gingerol is shown in Fig. 11. Here, at a lower pH, more than 60% of the drug was released within 24 hours, and nanoparticles were stable at this pH. On the other hand, at a higher pH, 6-Gingerol initially

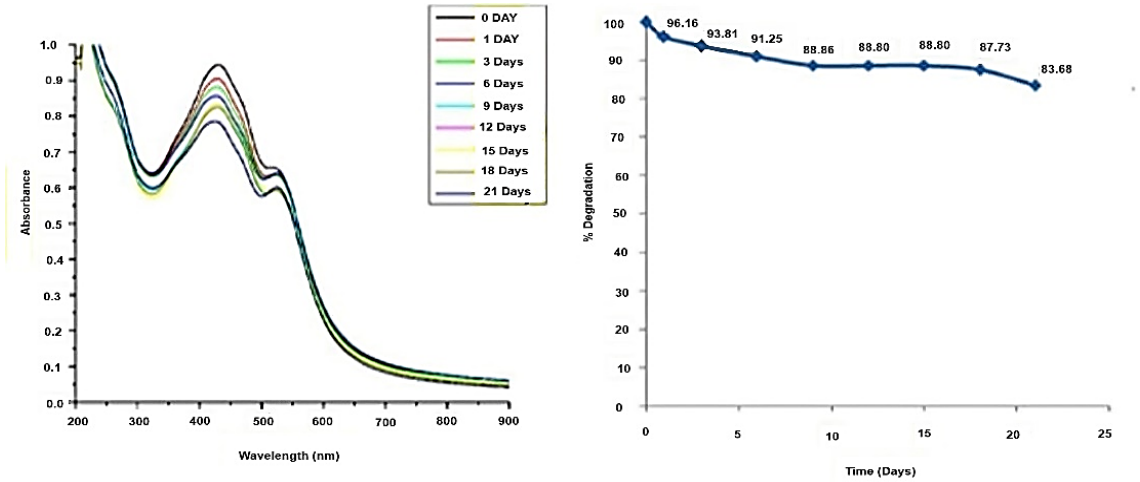

Fig. 9. Stability of AuNPs-6-Gingerol-PVP Conjugates in water

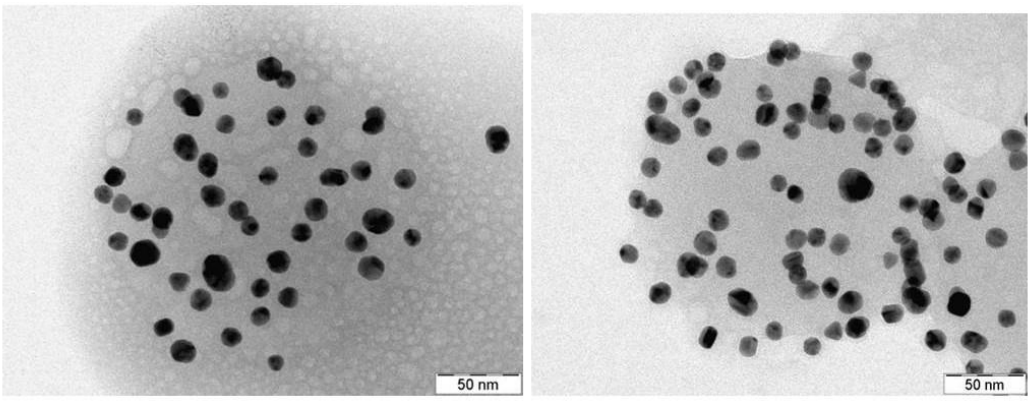

Fig. 10. Morphological changes of nanoparticles after drug release

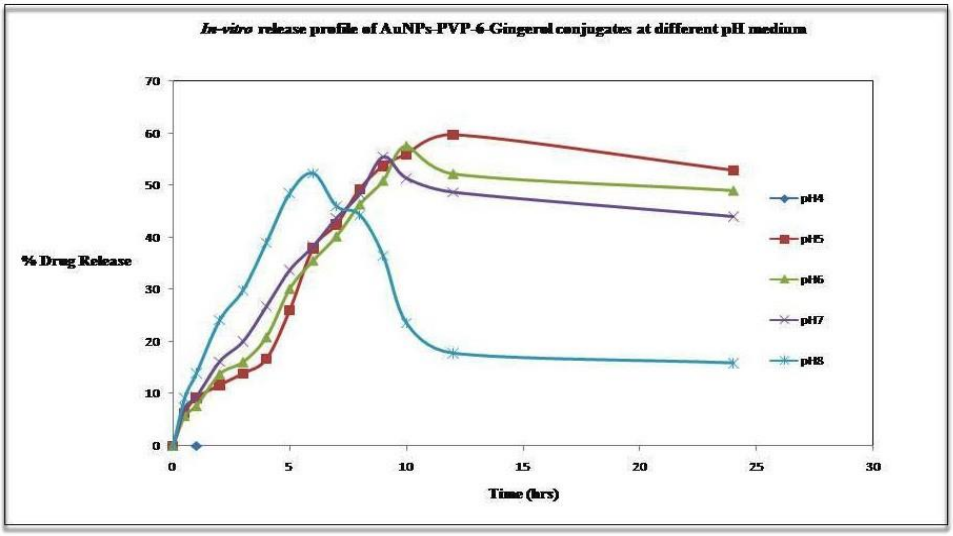

Fig. 11. *In-vitro* release profile of AuNPs-PVP-6-Gingerol conjugates at different pH medium

released very fast; more than 60% of the drug was released within 6 to 8 hours. After that, the intensity was gradually decreased. The reason behind that is the instability of 6-Gingerol at a higher pH. Therefore, the released 6-gingerol was degraded after 8 hours at a higher pH. The morphology of the NPs has changed with increased size and contrast after the release of 6-gingerol (Fig. 10).

4. SUMMARY

In the present study, an attempt was made to develop PVP-conjugated 6-GingerolNPs and AuNPs-PVP-conjugated 6-Gingerol nanoparticles for improving the bioavailability of 6-Gingerol. Development of 6-Gingerol nano delivery, by which we can improve the critical issues of 6-Gingerol pharmacokinetics. In our study, we synthesized two types of nanoparticles: the first is PVP-6-Gingerol NPs, in which 6-Gingerol facilitates the PVP to form a nanosphere, and the second is a metallic *cum* polymeric nanoparticle in which gold nanoparticles are adsorbed on the surface of the PVP nanosphere. In the second, the deposition of AuNPs on PVP nanospheres can be utilised as a promising novel drug delivery system as well as for the for the diagnosis of cancer cells. The advantages of improving the bioavailability of 6-Gingerol are that it reduces systemic side effects, maximises drug action at the desired site, decreases the metabolism of the drug before reaching the desired site of action, and decreases the distribution of the drug throughout the body, which may help in maintaining the required concentration at the desired site. Moreover, as nanoparticles have a higher carrier capacity, it helps to sustain and control action, thus reducing dose frequency and increasing patient compliance. The use of 6-

Gingerol in the treatment of cancer disease is helping to suppress proliferation, transformation, and metastasis of tumor cells and also act on the various stages of cancer cell development. In the current work, we prepared PVP-6-Gingerol polymeric and AuNP-PVP-6-Gingerol Metallic *cum* Polymeric nanoparticles. Thus, the developed formulations overcome the drawback and limitation of the conventional drug delivery systems.

5. CONCLUSION

In the present study, an attempt was made to develop nanoparticles, which are a conjugation of 6-Gingerol with polyvinyl pyrrolidone (PVP) and gold nanoparticles (AuNP), for improving the bioavailability of 6-Gingerol with a view to providing the desired solubility to the required site and achieving therapeutic efficacy. The process of chemical reduction has effectively prepared nanoparticles. It was possible to make the nanoparticles discrete and monodispersed. Polyvinylpyrrolidone is a biocompatible and biodegradable polymer used for preparing nanoparticles. FTIR was carried out to find out the possible interaction between the drug and polymer. The study revealed that there was an interaction site between the drug (6-gingerol) and polymer (polyvinyl pyrrolidone). The particle size analysis in HR-TEM was found to be in the range of 10–50 nm for gold nanoparticles, 200–300 nm for PVP-6-Gingerol nanoparticles, and 200–300 nm for Au-PVP-6-Gingerol nanoparticles. The surface charge or zeta potential of plain gold nanoparticles was found to be 4.06 mV, that of PVP-6-Gingerol nanoparticles was found to be -36.4 mV, and that of Au-PVP-6-Gingerol nanoparticles was found to be 50.1 mV. The low value of the zeta potential of gold nanoparticles shows the presence of flocs in nanoparticles, which leads to the rapid coagulation of particles. Therefore, conjugation with polyvinylpyrrolidone and 6-gingerol makes the nanoparticles more stable, and thus the nanoparticles show excellent stability for a long time after conjugation. The DLS size of nanoparticles was found to be 39.26 nm for plain gold nanoparticles, 220.5 nm for PVP-6-Gingerol nanoparticles, and 251.8nm for Au-PVP-6-Gingerol nanoparticles. The polydispersity index (PDI) of nanoparticles was found to be 0.622 for plain gold nanoparticles, 0.474 for PVP-6-Gingerol nanoparticles, and 0.191 for Au-PVP-6-Gingerol nanoparticles. The high value of PDI shows the polydispersity or presence of flocs in nanoparticles, which leads to the rapid coagulation of particles. Moreover, conjugation with 6-Gingerol and PVP makes the nanoparticles monodisperse, thus the nanoparticles show excellent stability after conjugation. From *in-vitro* studies, it was concluded that with an with an increase in pH of the dissolution medium, NPs release the drug very fast after that system was unstable because 6-gingerol is unstable at higher pH and shows the greatest stability observed at pH 4. So, it is showing excellent drug release at a lower pH. At pH 4 and pH 5, NPs release the drug by about 50 percent within 8–10 hours. From the percent drug loading, it was concluded that the maximum percentage loading was found to be 79.25% in the PVP-6-gingerol and 71.42% in the Au-PVP-6-gingerol nanoparticles. Stability testing of 6-Gingerol was carried out on a prepared 6-Gingerol conjugated nanoparticle, and it revealed that conjugated 6-Gingerol shows excellent stability compared to free 6-Gingerol because free 6-Gingerol is unstable in water. Morphological characterization of nanoparticles after drug release has been done by HR-TEM. The contrast of nanoparticles gradually increases with the release the drug as the size of the PVP nanosphere increases in both types of nanoparticles.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

CONFLICTS OF INTEREST

We declare no conflicts of interest or financial interests that the authors or members of their immediate families have in any product or service discussed in the manuscript, including grants (pending or received), employment, gifts, stock holdings or options, honoraria, consultancies, expert testimony, patents, and royalties.

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