Original Research Article

Synthesis and Characterization of 6-Gingerol Gold Nanoparticle (Au-6G-NPs)Conjugates for Improving Bioavailability

ABSTRACT

Introduction: In this study, an optimized conjugated gold nanoparticles were developed and investigated for improving the solubility and bioavailability of 6-Gingerol (Au-6G-PVP-NPs), an active and abundant component of *Zingiber officinale* with limited applications due to its poor water solubility plus lower oral bioavailability. 6-Gingerol is act as a cardiotonic & also used 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 cancercell development, but its less solubility makes it very challenging in therapeuticapplications.

Methods: In our study we have synthesized metallic*cum.* polymeric nanoparticle in which gold nanoparticle adsorbed on the surface of PVP nanosphere. In second step deposition of AuNPs on PVP nanosphere can be utilized as a promising novel drug delivery system as well as diagnosis of cancer cell & used as cardiotonic. The advantages of improving bioavailability of 6-gingerol is to reduce the systemic side effects, Nanoparticles were successfully prepared by chemical reduction method. 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.

Result and Discussion: The particle size analysis in HR-TEM and DLS were found in the range of 10-50 nm for gold nanoparticles and >200 nm for PVP-6-gingerol nanoparticles and 200nm for Au-PVP-6G Nanoparticle. The surface charge or zeta potential of plain gold nanoparticles were found to be -4.06, PVP and after PVP conjugation charge increased to -36.40mV and AuNPsPVP-6-Gingerol conjugates have shown -50.1mV. The high surface charge of final conjugates causes the greater stabilization of the nanoparticles. and thus, the nanoparticles show the excellent stability for long time after conjugation.The polydispersity index (PDI) of nanoparticles were found to be 0.622 for plain gold nanoparticles and 0.152 for PVP-6-Gingerol nanoparticles and 0.191 for Au-PVP-6-gingerol nanoparticle. Moreover, after

conjugation with 6-Gingerol and PVP makes the nanoparticles mono dispersed and thus the nanoparticles show the excellent stability after conjugation.

From *in-vitro* studies, it was concluded that increase in pH of the solution, it releases the drug very fast after that system was unstable, because 6-Gingerol is unstable in higher pH. So, it is showing excellent drug release in lower pH i.e. pH 4 and 5 and morphology of NPs system was also changed after the release of 6-Gingerol. Stability testing of 6-Gingerol was carried out from prepared 6-Gingerol conjugated nanoparticle and it revealed that conjugated 6-Gingerol shows excellent stability as compared to free 6-Gingerol because free 6-Gingerol is unstable in water.

Key words: *6-Gingerol, Gold nanoparticle,Polyvinylpyrrolidone,Au-6G-PVP-NPs*

INTRODUCTION:

Particulate dispersion materials or solid particles with a size range of 10-1000 nm are referred to as nanoparticles. The medication is encapsulated, dissolved, or bonded to the surface of a nanoparticle matrix.The merits of nanoparticles include controlling particulate size, surface characteristics and the release of pharmacologically active substances to obtain the therapeutically optimum rate and doses of site-specific action of the active material. [1].

Polymer-drug conjugates have a significant clinical effect by increasing the effect and dose of a number of pharmaceutical drugs that have previously been approved. However, their efficiency in drug loads may be limited by the number of conjugation sites in the polymer. A number of biocompatible polymerized nanoparticles have been developed in order to further improve drug loading capacity and incorporate spatial or temporal control on drug delivery. Polymer nanoparticles might also be conjugated to ligands which could permit drugs to be delivered spatially and temporally, increases the therapeutic effectiveness of drug products, and minimize their harmful side effects. A number of polymers have been utilized for the synthesis of polymeric nanoparticle like polyvinylderivatives, cellulose derivatives, polyethylene glycol etc. and modification in these polymers is also possible according to need of delivery, stability of therapeutic materialand type of synthesis [2].

For more than a century, scientists have been captivated by metallic nanoparticles, which are currently being utilized in biomedicine and research. These days, a variety of chemical functional drug groups can be added to these materials to enable their combination with antibodies, ligands, and pharmaceutically significant drugs. This opens up a wide range of potential biotechnology applications, including magnetic separation and pre-concentration target analytes, drug delivery targets, and gene delivery vehicles. Various imaging modalities, including MRI, CT, PET, ultrasound, SERS, and optical imaging, have also been developed to aid in the illustration of various disease states. These imaging methods vary in their techniques and equipment, and they require a contrast agent with particular physical and chemical properties. This resulted in the invention for use in these modes of imaging of several nanoparticular

contrast materials, such magnetic nanoparticles (Fe_3O_4), gold (AuNPs) and silver (AgNPs). In the development of novel multifunctional nano shells, several imaging methods have been created. [3]

Colloidal gold is a suspension (or colloid) of nanoscale particles of gold, known also as gold nanoparticles. However, colloidal gold solutions have different characteristics than bulk gold. The colloidal solution is therefore either an intense reddish color (less than 100 nm for particles), or a filthy yellow color (for larger particles). [4]

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 1951[13], like citrate mediated reduction. However, the most often used chemical technique of generating AuNPs was seed media growth reported in 1996 by Schmid. 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. [5]

Characteristic properties for gold nanoparticles include (a) small surface-to-volume ratio & small size (1-100 nm), (b) unique physical-chemical properties which can be changed in accordance with the size, composition, and shape requirements, (c) high strength shown by certain nano structural materials and (d) quantitative and qualitative target-binding properties. The larger surface area means that AuNPs have a distinct intrinsic reaction and hence the choice of material for the production of nanoparticles-base therapies is highly essential. [6]Nanomaterials can interact with biological systems in numerous ways depending on cell type, by means of various routes of absorption or the targeting of different organelles, by using their surface function and depending on the particle size, shape and aggregation state. Few types of nanoparticles have been approved for clinical trans-nanoparticle therapeutics are liposome polymeric(albumin) and metallic(Au) nanoparticles. [7]

Nanoscale structures show different physical and chemical characteristics on a molecular scale, compared with either small molecules or bulk materials, and find many applications in the field of biomedical and imaging therapy. Engineering Nanoparticles technology is one of biomedical research's fastest expanding fields.Hyperthermic radiotherapy, photodynamic thermal therapy, cancer drug carriers, bio labelling through electron microscopy to single particle detection, and photo-thermo-microscopy effectively used for nanoparticle. The interaction of nanomaterial and nanoparticles with the biological environment has an effect on its biological activity and it is essential for the proper concept of diagnostic and therapeutic nanoparticles to study carefully the nature of these interactions.[8] Recent developments in nanotechnology have led to the production of AuNPs used in a variety of biomedical applications,

including diagnostic assays.[9] The delivery of the genes and drugs to target tissues and tumors[10]and radiation therapy enhancers/sensitizers.[11]

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.[12] 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.[13] 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.[14]

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 systemsand subsides critical solubility issues.The advantages of improving bioavailability of gingerol is to reduce the systemic side effects, which 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. [15]

MATERIALS AND METHADOLOGY:

Formulation of gold Nanoparticles (AuNPs)

Citrate capped AuNPs synthesized by *J. Turkevich*method.[13]Chloroauric acid($HAuCl_4$) and tri-sodium citrate ($Na_3C_6H_5O_7$) used as a metallic nanoparticle precursorand reducing agent respectively. 100ml of 1mM $HAuCl_4$was boiled under refluxcondenser with continue stirring for 20 min. 10ml of 38.8mM tri sodium citrate wasadded directly into the boiled solution. Color of the solution has been changed from paleyellow to colorless and finally dark reddish within few minutes. [15]

Table 1: Formulation plan for Gold nanoparticles

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

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

Tri-sodium citrate and 6-gingerol both act as a capping and stabilizing agent. 100ml of3.88mM tri-sodium citrate in water heated under reflux condenser and 10mg polyvinyl pyrrolidone dissolved in 20 ml water and added in 100ml of tri sodium citrate solutionand boiled the solution for 45min. The solution was cooled and for the conjugation of 6-gingerol dissolved in 25ml of methanol and the solution added to

above solution, and the mixture of reactions that was stirred continues for 3 hours at 60^{o}C for the full removal of methanol from the solution with no reflux condenser.The PVP–6-gingerolconjugated solution then centrifuged at 12000 rpm for 40min at 4.0ºC to removeunattached 6-gingerol. This centrifugation has done three times to ensure that no free6-gingerol molecules were left in the final conjugate. The final solution used forspectroscopic study and TEM analysis. Part of this concentrated solution also will use forrelease rate analysis.[16]

Table 2: Formulation plan for PVP-6-gingerolNPs

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

Preparation of Master Formula: Conjugation of 6-Gingerol with PVP andAuNPs:

Polyvinyl pyrrolidone is used as a release retardant material. Prepared citrate capped goldnanoparticle heated for 10min, with addition of (10 mg PVP in 20ml water) PVP solutioninto the AuNPs solution and reaction mixture stirred continued for next 45min. For theconjugation of 6-gingerol 40mg of 6-gingerol dissolved in 25ml of methanol and the solution added to above solution, and the mixture of reactions that was stirred continues for 2 hours at 60^{o}C for the full removal of methanol from the solution with no reflux condenser.

AuNPPVP-gingerolconjugatedsolutionthencentrifugedat 12000rpm for40minat4.0ºCtoremoveunattached6-gingerol.Thisprocesshas done three times to ensure that no free 6-gingerolmolecules were left in the final conjugate. The final solution used for spectroscopic studyand TEM analysis. Part of this concentrated solution also used for release rate analysis.

Table 3: Formulation plan for AuNPs-PVP-6-gingerolnanoparticles

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

Characterization of Nanoparticles:[18-22]

The formulated nanoparticles were evaluated for chemical interaction, drug content uniformity, particle size andshape, drug loading, entrapment efficacy, surface charge, in-*vitro*drug release study and their stability. The prepared PVP-6-gingerol conjugates, goldnanoparticles and Au-PVP 6-gingerol conjugates

samples were characterized by UV–VisSpectroscopy (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 done in various pH condition, Surface charge of nanoparticles characterized by zeta sizer (Malvern), simultaneously PDI ofnanoparticles and drug loading calculated.

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 alwayshas 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 1mlconcentration was used for UV analysis.

b) FTIR Spectroscopy

Infrared spectrum of 6-gingerol, PVP and conjugated NPs were determined by using FTIR Spectrophotometer using KBr disks method for6-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 $cm^{-1.}$

Degradation of free 6-Gingerol and Conjugated 6-gingerol in water:

Degradation rate of free 6-gingerol and conjugated 6-gingerol in water studied for severalhours and days respectively. Sample was monitored with specific time intervals and %degradation of 6-gingerol has shown against time.

Morphology (Shape and Size):

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

Surface charge:

The zeta potential and surface charge of nanoparticles were determined by the ZetaPotential Analyzers. The zeta potential of a nanoparticle is commonly used tocharacterize the surface charge property of nanoparticles.

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:

$$PY(\%)=\frac{Nanoparticles weight}{}\times 100$$

$$Theoretical mass$$

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 givenpolymer sample. The PDI calculated is the weight average molecular weight divided bythe number average molecular weight i.e. PDI = Mw/Mn.

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.

$$DL(\%)=\frac{A(totaldrugwt)-\ A''(freedrugwt)}{A(totaldrugwt)}\ \times 100$$

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 [22]. 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 4: 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

RESULTS AND DISCUSSIONS

Critical solubility and bioavailability issues of 6-gingerol has been resolved byformulating its nanoparticle conjugates with gold nanoparticles (AuNPs) and polyvinylpyrrolidone (PVP) and each conjugation step

subjected to evaluation parameters likeparticle morphology, chemical interaction, drug loading and entrapment efficiency, Drugcontent uniformity and *In-Vitro* drug release study.

Characterization of AuNPs-PVP-6-GingerolNPs:

UV-Vis spectrum of conjugated nanoparticles

UV spectra provide the evidence of conjugation between AuNP-PVP-6 gingerol. In Figure 1 of UV spectra of a) 6-gingerol in methanol, b) citrate capped AuNPs, c) PVP conjugatedAuNPs, d) 6-gingerol conjugated PVP-AuNPs. The pure 6-gingerol and AuNPs, SPRabsorption peak shows at 218 nm and 275nm respectively, after conjugation of 6-gingerolwith PVP-AuNPs, 6-gingerol peak shifted to 228 nm and AuNPs -PVP conjugate peak shifted to280 nm due to the increasing the size after PVP capping.

Figure 1: UV-Vis Spectrum of Au-PVP-6-Gingerol conjugates in various stages

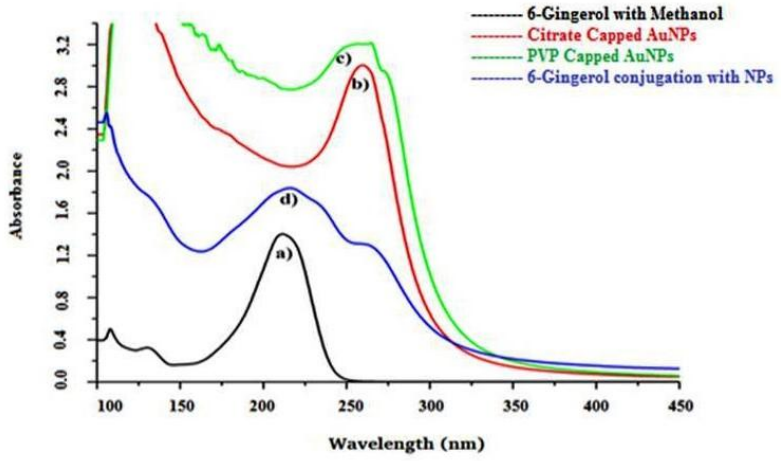

Figure 1 : UV-Vis Spectrum of Au-PVP-6-Gingerol conjugates in various stages

FTIR spectrum analysis:

From the UV-Vis spectroscopic evidence of 6-Gingerol conjugation, here also FTIRanalysis has done for conjugated nanoparticles and compared it with free 6-Gingerol andPVP. In Figure 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 4.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 at3512$cm^{-1}$and C=O group of PVP at 1656cm^{-1}remain same but these two functional group peak shifted to right side 3436$cm^{-1}$for 6-Gingerol and 1636cm^{-1}formation of a hydrogen bonding between OH- group of 6-Gingerol and C=O group ofPVP and both functional group responsible for binding.

Table-5: 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

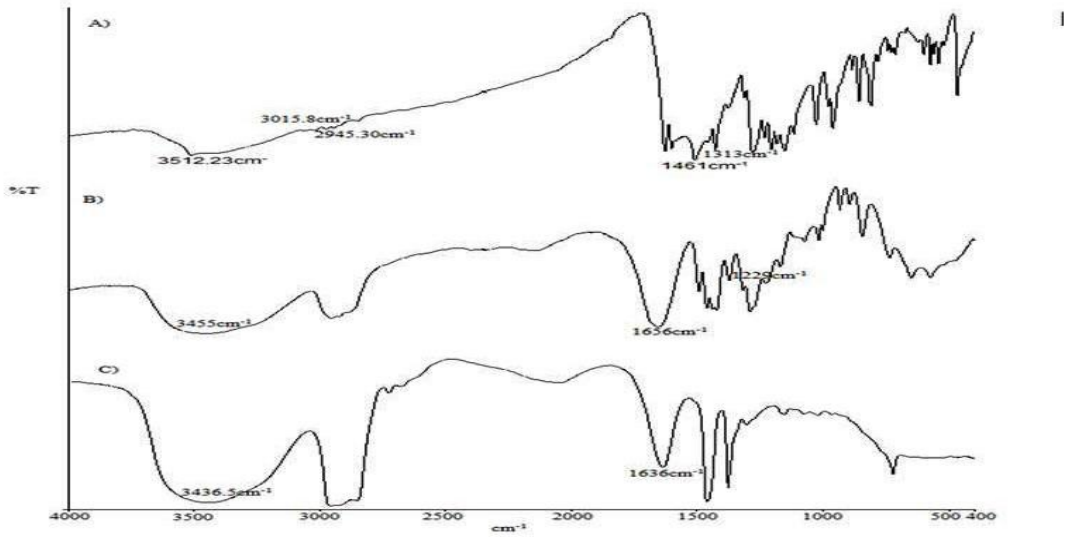

Figure 2: FTIR spectrum of a) 6-Gingerol, b) PVP capped AuNPs, c) Conjugated AuNPs-PVP-6- Gingerol

Morphological characterization:

The synthesized AuNPs were characterized for HR-TEM. The size of nanoparticles were around 10nm-50nm have shown in Figure 3(a) & (b). The AuNPs were also characterized for dynamic light scattering (DLS) and zeta potential. (Figure 4(a) & (b)) The hydrodynamic size of AuNPs was found to be around 39.26nm which is comparable with TEM size. The zeta potential gives charge on nanoparticle surface which was found to be -4.06mV. The value of zeta potential tends to under the range of strong agglomeration and precipitation. Which may be shows the solubilityissue of nanoparticle.

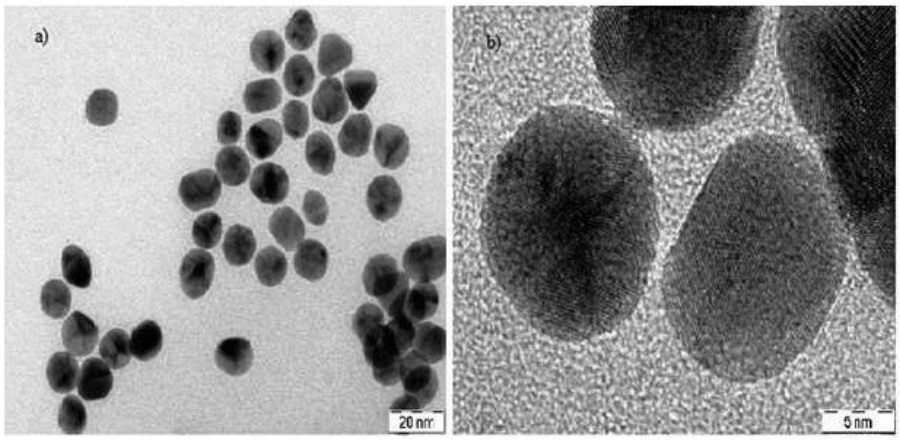

Figure 3 a) HR-TEM images of prepared AuNPs, b) magnify image of image

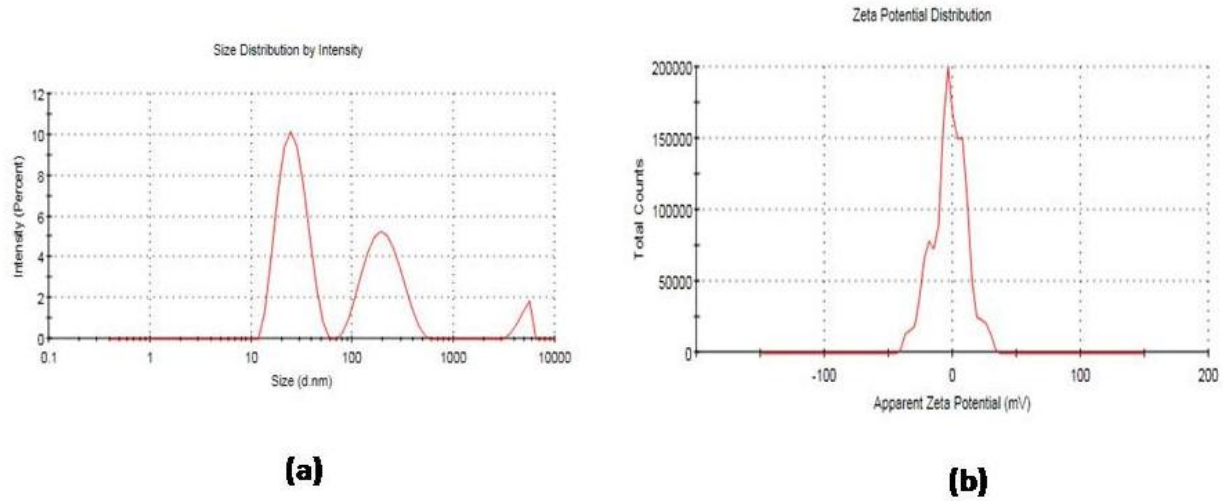

Figure 4: a) Zeta potential or surface charge and, b) average size distribution of AuNPs

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 NPssize is 200 nm (Figure 5a & b) but some are larger and smaller from this size range. Further size of NPs confirmed by the DLS study (Figure 6 a & b) and average size range of nanoparticle obtained 220.5nm. For zeta potential measurement water was used as a solvent for dispersing the nanoparticles. The zeta potential of the PVP-6-gingerol nanoparticles was -36.2mV. The high surface charge causes the greater stabilization of the nanoparticles.

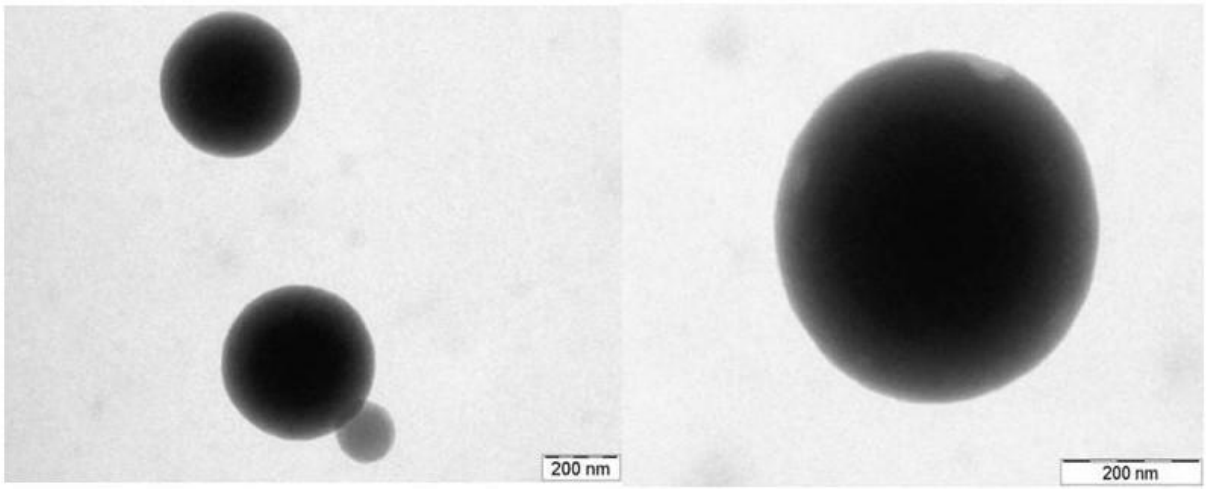

Figure 5:a) HR-TEM image of conjugated NPs, b) HR-TEM magnify images of conjugated NPs.

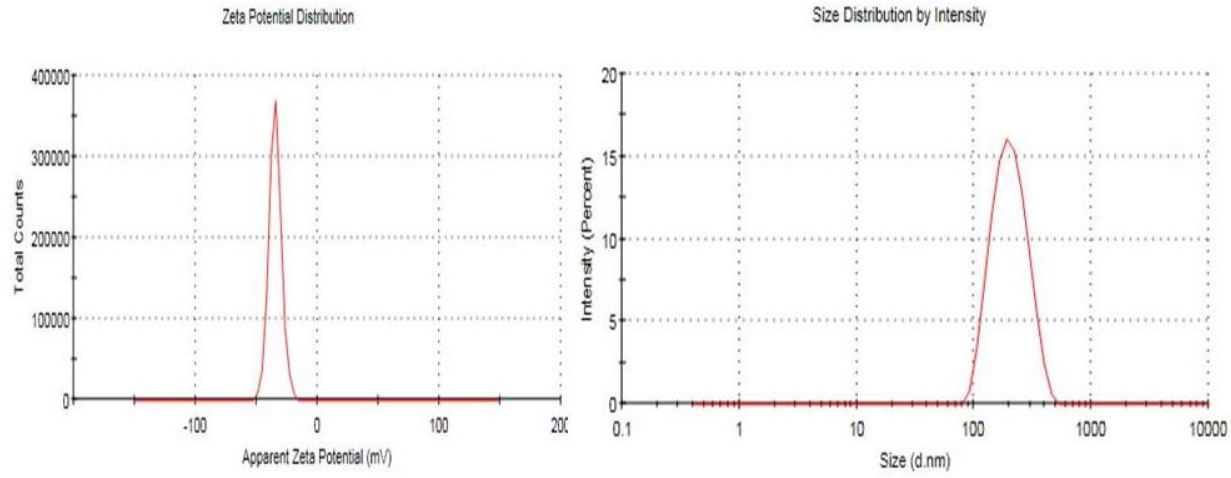

Figure 6: a) Zeta potential or surface charge, b) average size distribution of PVP conjugated NPs

Prepared AuNPs-PVP-6-Gingerol conjugated nanoparticles were spherical in shape areshown in Figure 7 - 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 10nm-50nm size range and contrast of the inner core and outer is same but after PVPcapping size slightly increase with different contrast in outer and inner part, after additionof 6-Gingerol, free PVP form a large nanosphere around 200nm and PVP conjugatedAuNPs and 6-Gingerol both attached to the surface of PVP nanosphere. Therefore, in finalconjugation (AuNPs-PVP-6-Gingerol.) most of the NPs size are around 200 nm (Figure 7 a, b, c,& d).The zeta potential of the AuNPs-PVP-6-Gingerolnanoparticles was -50.1mV. Further size ofNPs confirmed by the DLS study and average size range of citrate capped AuNPs was39.26nm, after PVP

capping size was increased to 220.5nm and final conjugate size was251.8nm obtained. (Figure8 a &b) For zeta potential measurement water used as a solventfor dispersing the nanoparticle. The zeta potential of the AuNPs was found to be -4.06mV, and after PVP conjugation charge increased to -36.40mV and AuNPsPVP-6-Gingerolconjugates have shown -50.1mV.(Figure 8) the high surface charge of final conjugates cause the greater stabilization of the nanoparticles.

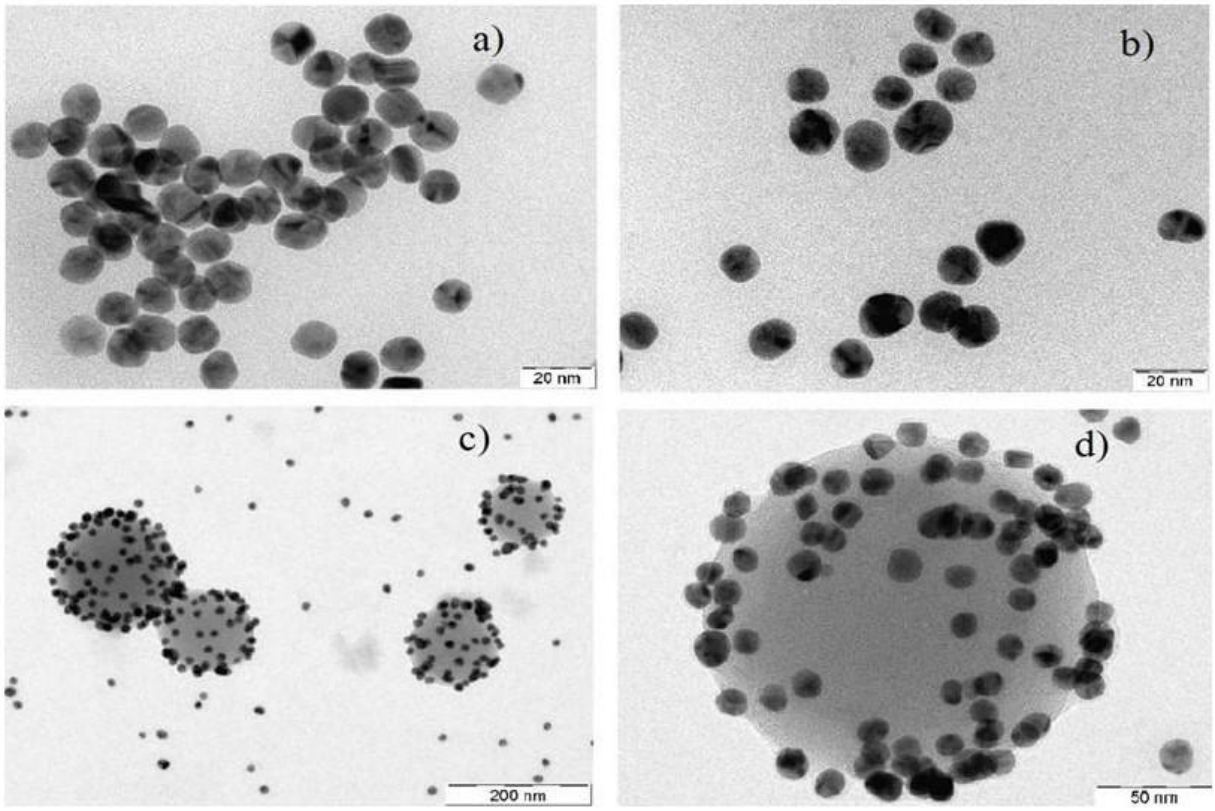

Figure 7: HR-TEM images of a) only gold nanoparticle, b) PVP capped Gold nanoparticle, c) Au- PVP-6-Ging. conjugate, d) magnify images of conjugated NPs.

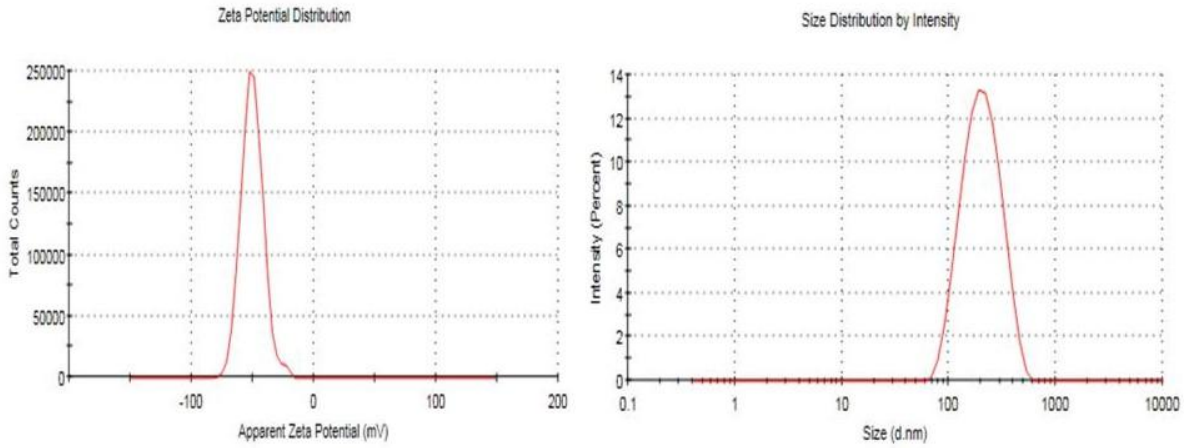

Figure 8: a) Zeta potential or surface charge and, b) average size distribution spectra of AuNPs-6-Gingerol-PVP conjugates

Table 6: 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

Drug loading and polydispersity index (PDI) of nanoparticles:

a) Drug loading was determined by centrifugation method. The redispersednanoparticles was centrifuged at 12000 xg 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%) wasdetermined using this equation.

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

$$A(total\ drug\ wt)$$

$$DL\% = \frac{40mg - 11.43mg}{40mg} \times 100$$

DL%= 71.42

b) Polydispersity Index(PDI)

Polydispersity gives the measurement of aggregation and agglomeration ofnanoparticle. PDI value of AuNPs-PVP-6-Gingerol NPs from DLS measurement was0.622 for AuNPs, 0.474 for PVP capped AuNPs and 0.191 for finalconjugate AuNP-PVP-6-Gingerol. Less PDI value of final conjugate concluded thatconjugated NPs were mono dispersed which facilitates its stability characteristicsfor long time.

Stability studies b/w free 6-Gingerol and conjugated Nanoparticles

It has already shown in Figure 9 the degradation of free 6-Gingerol in aqueousmedium, but after conjugation, the degradation rate of 6-Gingerol shown in Figure 9, in this stability study only 17% 6-Gingerol was degraded within 21days. Therefore, it can beconcluded that AuNPs-PVP binding with 6-Gingerol provided the greater stability to the6-Gingerol. Conjugated NPs also stable in aqueous medium for long time.

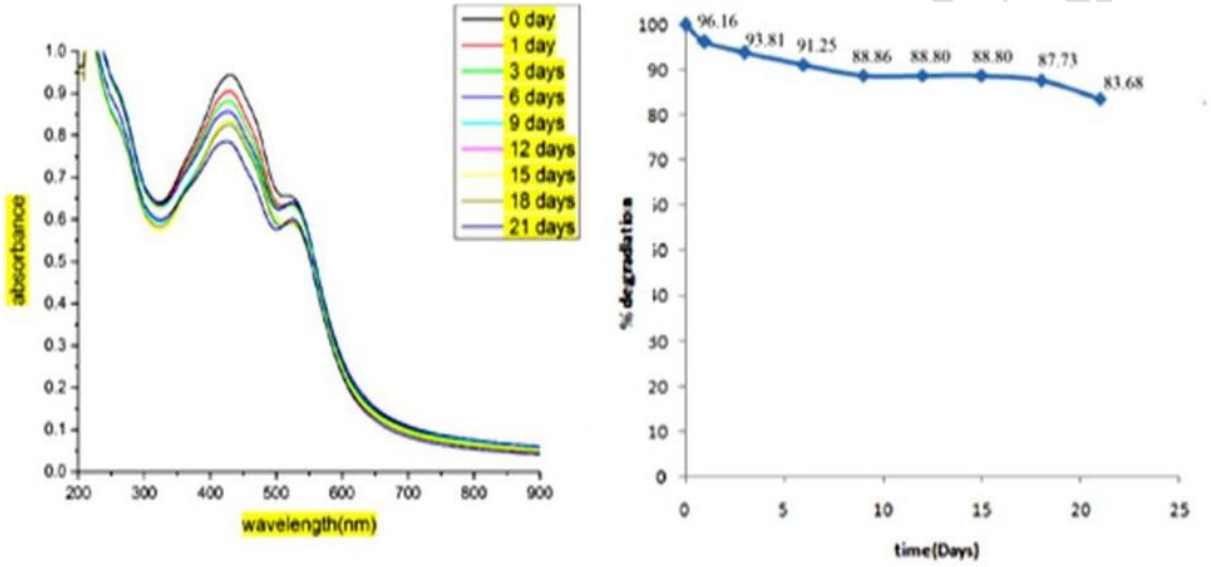

Figure 9: Stability of AuNPs-6-Gingerol-PVP conjugates in water

In-Vitro Drug release studies

The experiment was conducted in pH4, 5, 6, (phosphate buffer) and 8 (PBS) buffer at 35±0.5°C at gentle agitation, 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 are shown in figure11Here also at lowerpH more than 60 % was release within 24hr, and nanoparticles were stable at this pH, onthe other hand in higher pH,6-Gingerolinitially release very fast, more them 60 % drugwas release with 6 to 8 hrafter that intensity was gradually decreased, the reason behindthat is the instability of 6-Gingerol at higher pH. Therefore, released 6-Gingerol wasdegraded after 8hr in higher pH. Morphology of the NPs has changed with increase sizeand contrast after the release of 6-Gingerol (Figure 10)

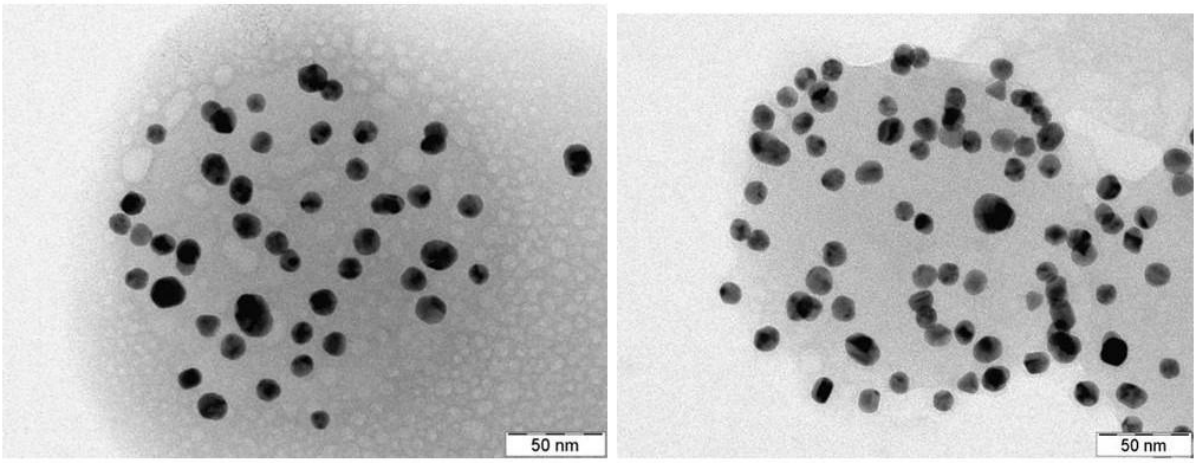

Figure 10 : Morphological changes of nanoparticles after drug release

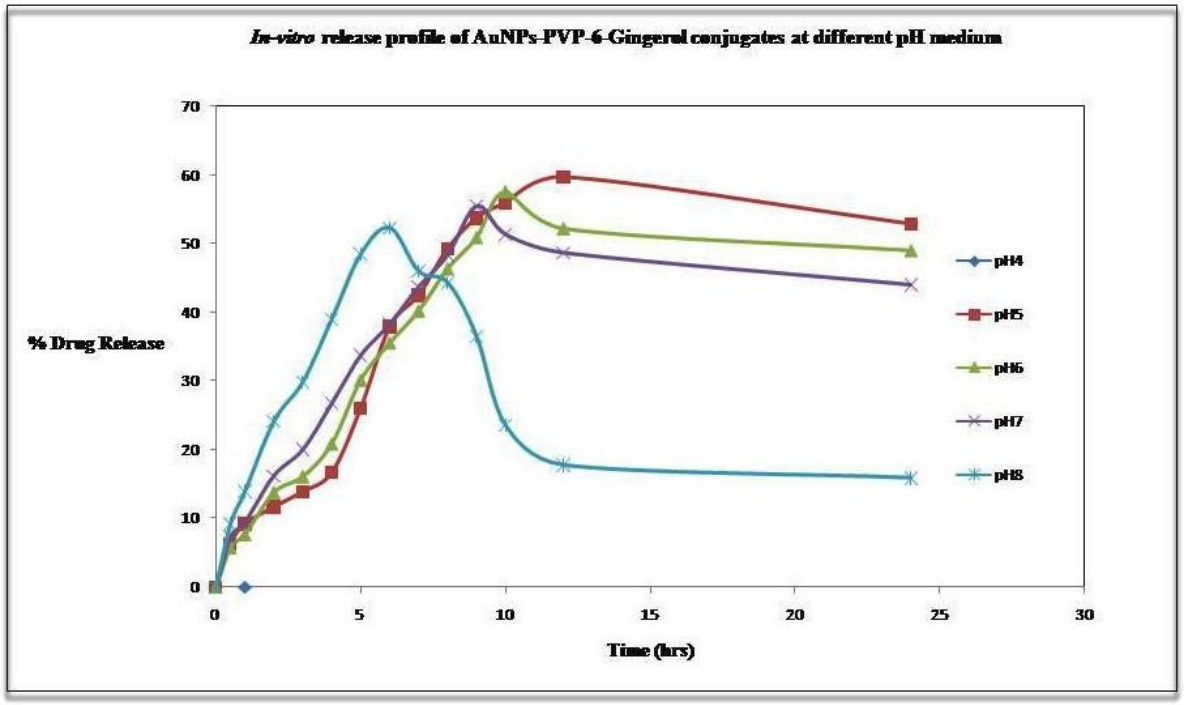

Figure 11: *In-vitro* release profile of AuNPs-PVP-6-Gingerol conjugates at different pH medium

CONCLUSION AND SUMMARY

In the present study, an attempt was made to develop nanoparticles which is aConjugation of 6-Gingerol with polyvinyl pyrrolidone(PVP) and gold nanoparticle(AuNP)for improving the bioavailability of 6-Gingerol with a view to provide desired solubility tothe required site and achieve therapeutic efficacy. The process of chemical reduction has effectively prepared nanoparticles. It was possible to make the nanoparticles discrete, monodispersed.Polyvinyl pyrrolidone 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 particles size analysis in HR-TEM were found to the range of 10-50 nm for goldnanoparticles and 200-300 nm for PVP-6-Gingerol nanoparticles and 200-300nm forAu-PVP-6-Gingerol Nanoparticle. The surface charge or zeta potential of plain gold nanoparticles were found to be-4.06mV, PVP-6-Gingerol nanoparticles was found to be -36.4mV, and Au-PVP-6-Gingerolnanoparticleswasfoundtobe-50.1mV.Thelowvalueofzetapotentialofgoldnanoparticlesshowsthepresenceofflocs in nanoparticles which leads tothe rapid coagulation of particles. Therefore, after conjugation with polyvinylpyrrolidone and 6-Gingerol makes the nanoparticles more stable and thus thenanoparticles shown the excellent stability for long time after conjugation. DLS size of nanoparticles were found to be 39.26 nm for plain gold nanoparticlesand 220.5 nm for PVP-6-Gingerol nanoparticles and 251.8nm for Au-PVP-6-Gingerol nanoparticle. The polydispersity index(PDI) of nanoparticles were found to be 0.622 for plaingold nanoparticles and 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 ofparticles. Moreover, after conjugation with 6-Gingerol and PVP makes thenanoparticles mono dispersed, thus the nanoparticles show the excellent stability after conjugation. From *in-vitro* studies, it was concluded that increase in pH of the dissolutionmedium, NPs release the drug very fast after that system was unstable, because6-Gingerol is unstable in higher pH and shows greatest stability observed at pH 4.So it is showing excellentdrug release in lower pH. At pH 4 and pH 5 NPs release the drug about more than50% within 8-10hr. From the percent drug loading, it was concluded that the maximum percentageloading 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 from prepared 6-Gingerol conjugatednanoparticle 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 nanoparticle after the drug release has done by HR-TEM. Contrast of nanoparticles gradually increase with release the drug withincrease the size of PVP nanosphere in both type of nanoparticles.

Summary:

In the present study, an attempt was made to develop PVP conjugated-6-GingerolNPs and AuNPs-PVP conjugated 6-Gingerol nanoparticle for improving the bioavailability of 6-Gingerol. Development of 6-Gingerol nano delivery by which, wecan improve the critical issues of 6-Gingerol pharmacokinetics. In our study we hadsynthesized two types of nanoparticles, first one is PVP-6-Gingerol NPs in which6-Gingerol facilitates the PVP to form a nanosphere, and another one is a metallic *cum*polymeric nanoparticle in which gold nanoparticle adsorbed on the surface of PVPnanosphere. In second one deposition of AuNPs on PVP nanosphere can be utilizedas a promising novel drug delivery system as well as diagnosis of cancer cell. Theadvantages of improving bioavailability of 6-Gingerol is to reduce the systemic sideeffects, maximizing the drug action on desirable site, decrease the metabolism of drugbefore reaching to desirable site of action, decrease the distribution of drugthroughout body, which may be helps in maintaining the required concentration at thedesired site. Moreover, as nanoparticles have higher

carrier capacity, it helps toprovide sustain and control action and thus reduce dose frequency and increases thepatient compliance. The use of 6-Gingerol in the treatment of Cancer disease is helping tosuppress proliferation, transformation, and metastasis of tumor cells and also act onthe various stages of cancer cell development. In the current work, we had preparedPVP-6-Gingerol polymeric and AuNP-PVP-6-Gingerol Metallic *cum* Polymericnanoparticle. Thus, the developed formulations overcome the drawback andlimitation of the conventional drug delivery systems.

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