BIOSCIENCES BIOTECHNOLOGY RESEARCH ASIA, March 2023. Vol. **20**(1), p. 127-134

Preparation and *In vitro* Characterization of Valerianic Acid Loaded Polymeric Nanoparticles

S. Imam Pasha, Aliya Meraj, Arshiya Meraj, Arwa Mohammed, Akifa Tanzil, Anam Fatima and Anupama Koneru

Sultan-ul-Uloom College of Pharmacy, Banjara hills, Hyderabad-500034, Telangana, India.

http://dx.doi.org/10.13005/bbra/3075

(Received: 01 September 2022; accepted: 25 January 2023)

This article describe the preparation of valerianic acid loaded HPMC nanoparticles (NPs) using the nano-precipitation method. Objective of this study include enhancement of the dissolution rate of the poorly water soluble drug(Valerenic acid obtained from Valerianawallichii), and further more to look into the in vitro properties, zeta potential and surface characteristics of nanoparticles (percent yield of nanoparticles, percent encapsulation efficiency and in vitro release characteristics).In phosphate buffer medium at pH 6.8, the cumulative drug release from the original drug and nanoparticles were up to about 12% and 35%, respectively. Valerianic acid release can be enhanced by the use of nanoparticles loaded with hydroxy propyl methyl cellulose (HPMC), that also enhances bioavailability and patient compliance.

Keywords: HPMC; In vitro release; Nanoparticles; Valerianawallichii; Valerianajatamansi.

Nanoformulations are unique drug delivery technique and are harmless and specifically breakdown in the gut..These nanoformulations enhance the properties of the valerianic acid(Torpey*et al*.,2018). Examples of some nanomaterials are polymeric nanoparticles, liposomes,nanosuspensions and nanoemulsions have been attempted as carrier vehicles to protect the herbal drugs from an external source of degradation, reduce their side effects and increase their bioavailability as well as used in targeted drug delivery (Ventola*et al*.,2012).Many studies reveal that nano delivery system can help to optimize the physiochemical properties of herbal drugs(Kashif*et al.,*2021) for its better action desired for the treatment of disease (Feng *et al.,*2020). The micro particles and nanoparticles drug delivery systems have a number of benefits over traditional dosing forms and used for treating both communicable and non-communicable diseases (Chopra *et al.,*2010).These include increased local drug concentrations, hydrophilic and hydrophobic drug loading, decreased gastrointestinal transit times, low interindividual variability, low risk of dose dumping, and reduced side effects. They can be administered via various routes, including oral, parenteral, and inhalation (Tania et al.,2004).The nanoparticle drug delivery system of size ranging from 1-1000nm is widely used in treatment and diagnosis of neurodegenerative and cardiovascular diseases(Bushra*et al*.,2020).

*Corresponding author E-mail: impazam@gmail.com

Valerianajatamansi,commonlyknown as *Valerianawallichii*. It is a rhizome herb of the *Valeriana* genus and belonging to the family Valerianaceae also called Indian Valerian or Tagara-Ganthodaand widely used in the indigenous system of medicine (Khuda*etal*.,2013). The herb *V.wallichii* is used in the treatment of insomnia, snake bites, nervous problems and can also be used as an analgesic (Nasir*et al*.,2018).The major active constituent of *V.wallichii* is valerenic acid(sesquiterpenes) which constitutes about 89.3% of the total herb(Mishra *et al*.,2012). The herb is native to India,Nepal, Pakistan and China. The valeriana rootshown in (Figure 1) have been used for hundred years from now as a sedative and antispasmodic and hence used for treating mild to moderate insomnia (Panjwani*et al*.,2012)and helpful for many patients suffering from sleepless nights due to stress disorders or any underlying disease conditions.Valeriana is an ancient tranquilizing drug used for many purposes like antispasmodic, psychotic, hypnotic,antibacterial (Tamanna*et al.,*2020), anthelmintic,cytotoxic and anticonvulsant activitiesdue to the presence of valepotriates and many other constituents.

The main purpose of thecurrent study was to prepare valerenic acid loaded HPMC, NPs using preparation method (antisolvent nanoprecipitation method) to enhance dissolution rate of poorly water soluble(Samantha *et al.,*2015) drug (valerenic acid) and minimizing its negative effects and researching the in vitro properties of nanoparticles like*in vitro* drug release in phosphate buffer(Kwok *et al*.,2011). The dissolution of the drug nanoparticles was significantly higher when compared with the pure drug in simulated intestinal fluid (phosphate buffer, pH 6.8) (Kumar *et al*.,2014).

MATERIAL AND METHODS

Materials

Tagaraformulation waspurchasedfrom Himalaya Wellness Pure Herbs, HPMC polymer and stabilizer (sodium lauryl sulphate) was purchased from S.D Fine Chemicals Ltd,Mumbai. Ethanol and Methanol were purchased from Agrizen Mills Private limited,Green lands,Hyderabad,Telangana. Standard buffer solution, phosphate buffer of pH 6.8 were prepared using potassium dihydrogen phosphate which was purchased from Chemipro labs, Nacharam, Hyderabad, Telangana, India and sodium hydroxide from Prime Laboratories.

Equipment used for the preparation includesUltra sonicator, mechanical stirrer at 1000rpm, sieves (mesh size 60 and 120) and USP basket type apparatusat50rpm.

Methods

Anti-solvent precipitation method

A technique called anti-solvent precipitation looks promising in producing ultrafine pharmaceutical particles.The basic idea is to dissolve the medication in a solvent, then mix the solvent solution with the medication to create an anti-solvent (in which the drug is insoluble). One of the most commonly employed synthesis techniques is the precipitation of nanoparticles from a solution. In the pharmaceutical and fine chemical industries, an anti-solvent precipitation approach is typically used to recover a product from solution inside a solvent where the product has high solubility. The core premise behind this is that the solid nanoparticles are retained by the filter mediumand form a filter cake while the liquid phase flows through to the filter medium and drains off as filtrate (Yana*et al*.,2014)

Preparation of valerenic acid loaded HPMC, NPs

Anti-solvent precipitation is a simple and quite effective approach to produce nanoparticles of poorly watersoluble drugs by mixing a drug solution and an anti-solvent(Dali *et al*.,2009).

Take 10g of drug powderof*V.wallichii*and HPMC (4g) in a beaker; to it add 15ml of ethanol, sonicated for 45 min using ultra sonicator.Anti-solvent solution was prepared by taking 1000ml of methanol, 3g of stabilizer (SLS) and 15ml of distilled water. Theanti-solvent solution was added to the drug solution after sonication and this was mixed homogenously by using mechanical stirrer at 1000rpm for 10 min. After sedimentation for about 2 h, filtered and dried by using Rotavapour.The dried powder was then passed through the sieve numbers 60 and 120 to get ultrafine drug loaded nanoparticles(Peltonen*et al*.,2018).

Characterization of NPs

In vitro release of valerenic acid from the NPs

Nanoparticle release investigations were carried out employing USP basket-type equipment. The formulation of 10 mg of ready NPs was placed in a basket suspended in phosphate buffer (pH

6.8), rotated vertically inside the vessel, and then heated to 37 0.5 °C and 50 rpm in a water bath. 5 ml samples were taken out of the release medium and replaced with 5 ml of brand-new phosphate buffer at the predefined time intervals of 5, 10, 15, 30, and 45 minutes. The samples were then placed in test tubes, and the amount of valerenic acid was determined using the trusted UV spectroscopic method at 280 nm. The standard graph (Figure 3) was created using the data using the same process as was used for the blank NPs (Table 2).

The percentage *in vitro* drug release(Nupur*et al*.,2020) of both the nanoparticles and the original drug extract were determined by measuring the absorbance (Dehuri*et al.,*2021) of the samples at its absorption maxima (280nm) by using UV spectrophotometer (Jin *et al*.,2015) and the graphs were plotted using the data obtained*in vitro* release patternis shown in (Figure.2). Dissolution data showed that 35% of valerenic acid was released from polymeric nanoparticles within 45 min (Table 1).

Fig. 1. Picture depicting roots of *V.wallichii*

Table 1. Percent drug release of nanoparticles and original drug v/s time

time (min)	Per cent drug release of valerenic acid loaded nanoparticles	Per cent drug release of original drug
0	0	0
5	10.542	3.4445
10	10.726	5.5513
15	11.58	7.8831
30	32.715	11.933
45	32.96	12.915

Determination of percentage yield and encapsulation efficiency of valerenic acidnanoparticles

The original drug and the nanoparticles were separated through filtration by filter paper. The nanoparticles was collected on the filter paper,dried and weighed.The percent yieldand the percent encapsulation efficiency (EE %) was calculated and was 40% and 56.5% respectively.

Morphology of the valerenic acid loaded nanoparticles using SEM

The manufactured nanoparticles were observed using a scanning electron microscope (SEM), and the images showed that they had a roughly spherical morphology and were nano-sized. This method was used to determine the particle size of the prepared nanoparticles (Figure 4).The particle size affects the biopharmaceutical properties of the nanocarriers as well as drug release and hence should be considered. The particle size of the nanoparticles was determined and the particle size of particles varied, the range was found to be 120-235 nm.

Zeta potential

Zeta potential measures and quantifies the effective electric charge on a nanoparticle's surface. A web surface charge on a nanoparticle is caused by the concentration of ions with opposing charges close to the nanoparticle surface. These layers of dipolar ions move both individually and collectively with the nanoparticle. The zeta potential's magnitude gives sequential information regarding particle stability. The stability increases as the magnitude of potential increases.

High Brownian motion is represented by small particles with a high zeta potential. Aggregation does not therefore develop to improve

Table 2. Standard graph of valerenic acid loaded nanoparticles

Concentration (mcg/ml)	Absorbance
2	0.02
4	0.04
6	0.061
8	0.085
10	0.104

the stability of the dispersion system. When zeta potential is low, attraction outweighs repulsion, causing dispersion to break down for particle flocculation to take place. With a positive charge, 13.9 0.1 mV of zeta potential is generated. Using a commercial zeta sizer, the zeta potential of the polymeric nanoparticles was measured. The zeta potential value thus obtained was enough for the product's physicochemical stability.

RESULTS

Valerenic acid loaded nanoparticles were prepared by using the nanoprecipitation method and the profile of *in vitro* drug release was given in (Figure 2).The dissolution studies were performed using USP basket type apparatus using 6.8 pH phosphate buffer at 50rpm and the 5ml of samples were withdrawn at 5,10,15,30,45 min and the absorbance of each sample was measured using UV spectrophotometer, graphs were plotted from which the concentration of drug release at respective time intervals was determined. All the release studies were conducted under sink conditions because a non-sink condition could substantially underestimate the drug release from nanoparticles. Hence, the solubility of drugs in the release medium was determined prior to the release studies. Therefore, dissolution profile of the original drug and the drug loaded nanoparticles was compared and the dissolution profile of the nanoparticles was found to be greater than the original drug(Rainbow*et al*.,2004).Hence, the valerenic acid loaded nanoparticles have an

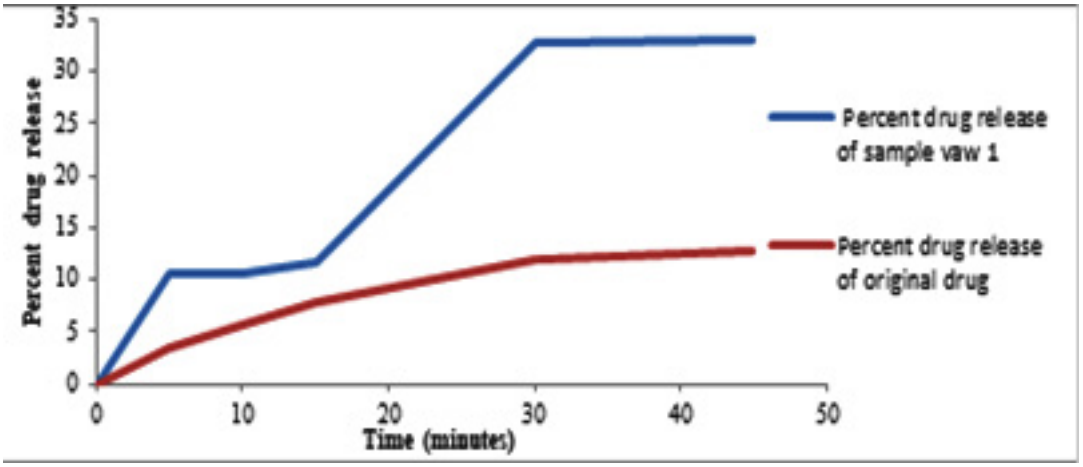

Fig. 2. Cumulative drug release

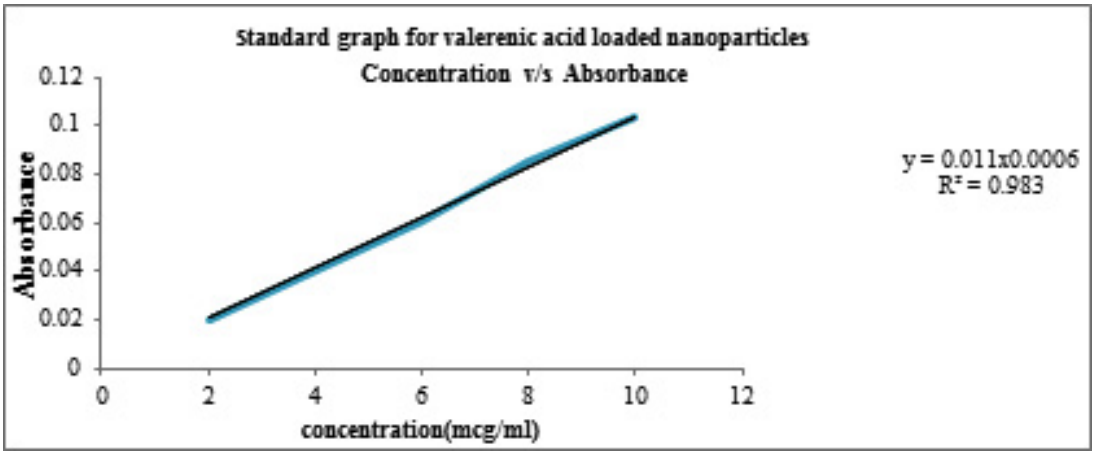

Fig. 3. Standard graph for valerenic acid

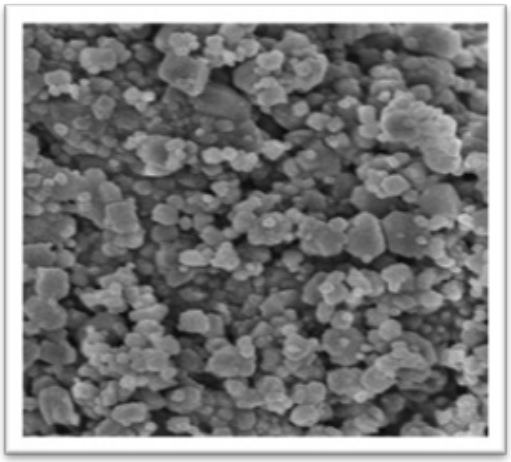

Fig. 4. SEM image of valerenic acid loaded polymeric nanoparticles

enhanced dissolution profile and helps in faster drug release as well as rapid drug action (Dinesh *et al* .,2020).

DISCUSSION

The most popular herbal remedy for reducing insomnia (sleeplessness/restlessness) is valerian root, which is a safe and efficient alternative to allopathic sleeping pills, which can be addictive and have memory and attention problems. They are typically not advised for the long-term treatment of sleeping disorders, and these medications also impair cognitive function ,alertness and cause prolonged drowsiness, whereas *V.wallichii* solves all these issues. The inventive techniques for anti-solvent nanoprecipitation suggested in this work appears to be an intriguing strategy to enhance the dissolving property of the *V.wallichii* herbal dosage form, as could be seen from the dissolution curves.

The *in vitro* release study, which is a crucial step in determining the safety, effectiveness, and quality of a drug delivery system based on nanoparticles. The, development of *in vitro in vivo* correlation of polymeric nanoparticles in the near future may benefit from this enhanced analytical technique for evaluating *in vitro* drug release from polymeric nanoparticles.

The *in vitro* release kinetics of nanoparticles provide critical information regarding their ability to modify the drug release characteristics . In this study, we use polymer in the preparation of the nanoparticles as the polymeric nanoparticles which is the mainstay of the nanoparticle type because of its extensive medical applications, high biocompatibility and relative stable drug release.

To lessen these immediate release dose form problems, modified release mechanisms have been created (Song et al.,2020). Particularly appealing for the preparation of various medication formulations in the pharmaceutical industry is the design of nanosystems. Nanoparticulate drug delivery systems have a significant potential to regulate drug release rate, decrease drug plasma concentration fluctuations and side effects, increase therapeutic efficacy, increase drug stability, decrease drug dosing frequency, and protect the drug from metabolization and degradation (Lijo et al.,2020).

In the development of polymeric nanoparticles for both hydrophilic and hydrophobic medicines, HPMC polymers are most commonly implemented. It possesses good mechanical qualities, predictable biodegradation tendencies, and great biocompatibility (Mohd et al.,2021). In terms of immunogenicity and toxicity, HPMC exhibits a minimal risk and thereby reduce the side effects possible. In this study, the nano precipitation method was used to create the valerenic acid loaded nanoparticles.

Moreover, the nano precipitation is based on the rapid diffusion of an organic solvent into an aqueous medium in the presence of sodium lauryl sulphate, followed by the interfacial deposition of a polymer.

The herbal formulation of V.wallichii was reported to have several activities in treating neurological , psychological and digestive disorders. Therefore it is used in treating disturbed sleep (insomnia). However for the oral administration of the herbal drug it has low bioavailability and a short half life , hence the use of delivery system such as polymeric nanoparticles ,generally increases the drug dissolution of the active drug (valerenic acid). *invitro* drug release studies in the phosphate buffer of pH 6.8 showed enhanced drug dissolution in case of the polymeric nanoparticle of V.wallichi when compared to the herbal drug formulation.

To create smaller-sized particles, ultrasonication or high-speed homogenization is performed. Even though the amounts of the

original drug and the nanoparticles were the same when the dissolution trials were being conducted, the organoleptic qualities were improved when nanoparticles were utilised as drug carriers, and the drug release rate also rose from 12% to 35%.

One of the most important applications of polymeric nanoparticles is sustained and controlled delivery of drugs and maximum therapeutic action and they stand out as a key tool to improve drug bioavailability , reduce the toxicity of drug as well as help in the site specific drug action. Various factors like solubility of drug, desorption, drug diffusion or erosion can affect drug release.

It was discovered that the release kinetics of herbal formulation and polymeric nanoparticles in sink circumstances were improved through comparative investigation of the dissolution curves.

In overall, the major goals of this study are(1)to study the release properties of the herbal drug formulation and compare it with the relase profile of the prepared polymeric nanoparticels. (2) to validate the anti-solvent nanoprecipation method , ultrasonication method and *in vitro* dissolution methods ,in which the cumulative drug release of the herbal drug and the nanoformulation were compared and it proved that there was enhancement in the drug release by 23 %.(3) to evaluate the prepared polymeric nanoparticle by scanning electron microscopy through which the particle size of the roughly spherical nanoparticles was found to be in the range of 120-235nm, zeta potential .

Examples of various other drugs studied similarly for their *in vitro* drug dissolution, are as follows:

1. In a study, Preparation of herbal nanoparticle loaded with piperene with0.2%PVA polymer and other nanoparticle without the polymer and a comparitive dissolution study was performed and it was concluded that preparation of herbal nanoparticle loaded with piperine gives better release with 0.2%PVA and without polymer loaded drug does not give an excellent result.

2. In another study of nifedipine (NF) (anti-hypertensive drug)loaded PLGA nanoparticles(Y I Kim et al.,1997 these systems were used for sustained release of drug ,also reduce their side effects and increase patient complaince. The nanoparticles were prepared by two different techniques (1)nanoprecipitation method-N2 and (2) emulsion solvent evaporation method-N4. The extents of cumulative drug release from N 2 and N4 in phosphate buffer with pH 7.4 medium were up to about 100% in 38 days and 22 days respectively.

3. Curcumin loaded polymeric nanoparticles were prepared to enhance the bioavailability of the hydrophobic drug. The nanospheres prepared was studied for 10 days and usually the drug release occurs in biphasic manner, with initial burst phase followed by a diffusion controlled slower drug release phase . Firstly initial phase was about 10 to 13% and then the diffusion phase was about 65% release of drug.(Xiao-peng et al.,2019).

4. The *in vitro* release study of pure quercetin and polymer PLGA nanoparticles of quercetin indicated that the PLGA nanoparticles of quercetin sufficiently entrap quercetin in the solid PLGA nanoparticles and maintained sustained drug release of quercetin in comparison with pure quercetin around 20% was released and in PLGA nanoparticles 65% in phosphate buffer pH 7.4 at 37degree celcius ,in 24 hours.(Md.khalid et al., 2016)

Therefore in the current study, it is validated that the polymeric nanoparticles were prepared and evaluated for various characteristics and hence it was seen that the polymeric nanoparticles prepared are more efficient in the drug release profile when compared to the herbal drug formulation of V.wallichi.

CONCLUSION

As a result, valerenic acid-loaded HPMC nanoparticles were created in the current work using the anti-solvent precipitation approach. In phosphate buffer with a pH of 6.8, the amounts of cumulative drug release from the original drug and the valerenic acid nanoparticles were around 12% and 35%, respectively. These nanoparticles can be effective methods for enhancing valerenic acid's solubility profile, lowering its side effects, and enhancing patient compliance.

Formulation of herbal drugs in nano-carrier is a promising direction for the development of primary treatment and a promising suggestion for many pathological illnesses. Nanoherbal systems therefore have positive qualities for increasing activity and treating insomnia with plants. Therefore, the use of nano-carriers in

herbal treatments will increase surface area, improve solubility, and make drug targeting easier. Therefore, the integration of nanotechnology with herbal supplements offers a link between the science of plants and nanotechnology, resulting in a minimum application and the production of toxic chemicals that kill living things. As a result, many nanoparticles have the potential to produce herbal medications with improved therapy.

ACKNOWLEDGEMENT

We are thankful to the Principal and Management of Sultan-ul-Uloom College of Pharmacy,for providing us all the materials, equipmentrequired for completing this research.

Conflict of Interest

Authors declared that there is no conflict of interest relevant to this article.

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