

ORIGINAL ARTICLE

Preparation, characterization, in vitro antibacterial activity and comparative pharmacokinetic study of orally administered curcumin loaded PLGA nanoparticles

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Abstract

Curcumin is used as nutraceutical. It is a yellow pigment derived from turmeric (*Curcuma longa*). It can be used as anti-diabetic, antibacterial, anti-tumor, anti-rheumatic, anti-viral and anti-cancer agent. But poor solubility, rapid degradation and low bioavailability have limited its use in clinical practice. The best way to overcome these problems is to load curcumin in poly lactic co-glycolic acid (PLGA) nanoparticles. Curcumin loaded PLGA nanoparticle increase the bioavailability and absorption of drug. The focus of study was to introduce a method for preparation of nanoparticle with a view to increase the aqueous solubility of curcumin, and to characterize the prepared nanoparticles and access their antibacterial activity and to modify the pharmacokinetic behavior of drug. The nanoparticles were prepared by nanoprecipitation method and it was found that the prepared nanoparticles lies within nanometer range. The evaluation of in-vitro release of curcumin loaded nanoparticle showed that formulation possess high drug loading capacity and entrapment efficiency (89.4%). A minimum inhibitory concentration of curcumin was determined for a variety of bacterial strains and was compared to that of commercially available antibiotic ampicillin. It was found that value of MIC of curcumin was much more pronounced than ampicillin against *Staphylococcus aureus* and *Escherichia coli*. The pharmacokinetics parameters were calculated by the pharmacokinetic APO software. The results of comparative pharmacokinetics of these nanoparticles in comparison to the pure standard showed that AUC of Cur NP was 95.38 h.mg/L much greater than pure curcumin (13.6 h.mg/L). The value of C_{max} for Cur NP was also increased to 12.50µg/ml as compared to 1.50µg/ml of curcumin alone. Similarly, the value of T_{max} was also increased. The increase in C_{max} and T_{max} values after administering curcumin loaded PLGA nanoparticles can be explained by controlled and sustained release of curcumin from PLGA nanosystem.

Keywords

Curcumin
Nanoparticles
Pharmacokinetic
Rabbits

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Introduction

In the recent years the most dangerous threat to the public is dissemination of resistant microbial strains and the main cause behind this is excessive and rapid use of

antibacterial. Due to repeated and increased use of antibiotics there is an increase growth of resistant bacterial strains. Some other factors that may worsen the problem of antimicrobial resistance are the wrong prescription of antibiotics for viral diseases, in feeds of

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livestock the generalized use of antibiotics and negligence of a person in completing the therapy. To avoid this resistance scientists are working on some alternate therapies. (Suzuki *et al.*, 2016).

A change in food habits and a new life style of present era has led to emergence of a number of diseases. And people are not happy with highly expensive treatment of disease in modern medicine and also their side effects and they need an alternative treatment with more beneficial effects. These circumstances led to use of nutraceuticals. The word nutraceutical is obtained from two terms “pharmaceutical” and “nutrition”. They are explained as food or portion of food that play a chief role in alteration of normal physiological function to maintain human’s health (Das *et al.*, 2012).

Curcumin is a pigment that is first discovered by Vogel and Pelletier in 1850 about two centuries ago. It is yellow in color and present in turmeric which is an Indian spice. It has a potent antimicrobial effect and is a nutraceutical. This agent performs its antimicrobial action at molecular level by suppressing several cell signaling pathways including ROS, COX-2, NF- κ B, STAT3 and Nrf2. (Kunnumakkara *et al.*, 2017).

Despite of the fact that curcumin is safe and efficacious, its clinical uses are restricted because of its poor bioavailability. The factors that may result in low level of curcumin in plasma and tissues are its reduced absorption, rapid metabolism and rapid systemic elimination. Drug effect or bioavailability can also be altered by physical and chemical properties like hydrophobicity, Pka of drug and solubility (Xie *et al.*, 2012).

One of the major drawback in use of curcumin is its decreased oral bioavailability in vivo and reduced solubility in aqueous solvent. Because the bioactive constituents of turmeric (curcumin and turmeric oil) have hydrophobic natures so turmeric is given in form of emulsion in oil or milk. Curcumin show interaction with surfactant, phospholipid and dextrans. Scientist are discovering various methods to increase its delivery like its incorporation into phospholipid vesicles this method is use to administer curcumin intravenously into splenic macrophages and bone marrow and other method is incorporation of curcumin into liposomes.

However, scientists are also working on methods to increase the oral bioavailability and absorption of curcumin one method is to deliver curcumin in form of polymer-based nanoparticles (Anand *et al.*, 2009). There is no published report on pharmacokinetics of curcumin loaded PLGA nanoparticles in rabbits so we conducted this study to see the behavior of this nanoformulation in in vivo system.

Materials and Methods

Chemicals: Curcumin and PLGA were purchased from Sigma-Aldrich, polyvinyl alcohol (PVA) was purchased

from Duksan pure chemicals. Dimethyl sulfoxide, methanol, ascorbic acid, acetic acid, acetone were purchased by Merk. Nutrient broth was purchased from lab M.

Preparation of curcumin loaded nanoparticles: Cur PLGA nanoparticles were developed by following solvent displacement method with little changes. First of all, 20mL PVA solution was taken in a beaker and put it on magnetic stirrer under 120rpm at 25°C. Then, by using micropipette PLGA and curcumin solution was added in PVA solution drop by drop slowly and steadily for homogenous distribution of drug and polymer in the formulation. As a result, dispersion of nanoparticles was prepared. (Pool *et al.*, 2012).

After freshly preparing the dispersion of nanoparticles, it was kept on magnetic stirrer for continuous stirring for 4 hours at 40°C, so that the organic phase (acetone) was completely removed. The remaining dispersion was then centrifuged at 2500rpm for half an hour at 25°C. The pellet of nanoparticles was settled down. The supernatant was discarded. Then the settled down pellet was dispersed in distilled water on vortex mixer. The dispersion was sonicated for 10 minutes for complete homogenization. (Gao *et al.*, 2010).

Characterization of curcumin loaded PLGA nanoparticles: Zetasizer Nano-ZS90 was used to measure poly dispersity index, particle size and zeta size of curcumin loaded PLGA nanoparticles.

Determination of encapsulating efficiency: For determination of encapsulating efficiency firstly prepared a stock solution by dissolution of 10mg of curcumin in 100ml methanol. The maximum wavelength (λ_{max}) of curcumin was determined by taking wavelength scan at UV-visible spectrophotometer. Standard curve was generated with the help of different dilutions of pure curcumin for determination of % encapsulation. The given formula is used for determination

% Encapsulating Efficiency = (Curcumin weight in nanoparticles)/(Initial weight of curcumin used)*100

In vitro determination of minimum inhibitory concentration of CUR NP: Using broth dilution method with minor modifications, minimum inhibitory concentration of curcumin loaded PLGA nanoparticles was determined. Stock solution of curcumin loaded PLGA nanoparticles was prepared in 10% solution of dimethyl sulfoxide (DMSO). Then different dilutions of curcumin loaded PLGA nanoparticles from a concentration of 1mcg/mL to 500mcg/mL were prepared. In each dilution, 0.1 mL of tested strains (Staphylococcus aureus, Escherichia coli) of inoculum was added and was incubated for 24h at 37°C separately in all the previously prepared dilutions of Cur PLGA nanoparticles. Presence of turbidity showed growth of bacteria. The concentration at which there was no growth

of tested bacteria (no turbidity) was regarded as MIC of curcumin nanoparticles (Jaisinghani 2017).

Determination of comparative pharmacokinetic study of simple curcumin and curcumin loaded PLGA nanoparticles: Six rabbits (n=6) were taken from animal house of University of Agriculture, Faisalabad. Suitable environment and adequate food was provided to all the rabbits for one week before testing. After one week, each rabbits were weighed. Then according to their weight 15mg/kg pure curcumin dose in distilled water was given orally to each rabbit. Then blood samples were collected from all the rabbits at 0h, 0.25h, 0.5h, 1h, 2h, 4h, and 8h, 12h, and 24h intervals in heparinized, EDTA containing vials. Pretreatment of all the collected blood samples was done and then they were analyzed by HPLC/UV-visible spectrophotometer analysis.

Then again suitable environment and adequate food was provided to all the rabbits, for one week for washing out pure curcumin, before further testing. After one week, each rabbits were weighed again. And given 15mg/kg of Cur NP dose and again blood samples were taken. The blood samples were analysed in same way as mentioned above. (Biasutto et al., 2010).

The concentration of curcumin in blood was determined by taking 1mL blood from each dilution in mini test tubes. 0.01M solution of ascorbic acid and 0.6M solution of acetic acid were prepared. Then 0.1mL of ascorbic acid solution and 0.1mL of acetic acid solution was included in all mini test tube and was shaken well. After that acetone 4mL was added in them and was sonicated for 2 minutes. Then all the samples were centrifuged at 3000rpm for 5 minutes. Supernatant was collected and then sent the supernatant for UV-visible spectrophotometric analysis (Biasutto et al. 2010).

Determination of pharmacokinetic parameters: The Pharmacokinetic parameters were determined by linear regression method using APO software version 3.02.

Results

Nanoparticle's characterization: The physical and chemical properties like zeta potential, entrapment efficiency, zeta size and polydispersity index of nanoparticles effects the behavior of nanoparticles-based drug delivery. The diameter of prepared formulation was found to be 420.5nm and polydispersity index was 0.36 as given in Figure 1 and Table 1 respectively. The zeta potential value was -1.35mv as given in Figure 2.

Entrapment efficiency: Entrapment efficiency was calculated by drawing the standard curve, utilizing UV-visible spectrophotometric analysis at 402nm wavelength which was represented in Figure 12 and then by putting values in the given equation. The encapsulation efficiency of the prepared formulation calculated by given formula was 89.6%.

$$\% \text{ Entrapment} = \frac{\text{Curcumin weight in nanoparticles}}{\text{Initial weight of curcumin used}} * 100$$

Table 1: Comparative Pharmacokinetic parameters of pure Curcumin and Curcuminloaded PLGA nanoparticles.

Parameter	Unit	Curcumin	Cur NPs
		Mean±SEM	Mean±SEM
AUC	h.mg/L	13.605±0.64	95.38±0.891
Clearance	L/h	1.34±0.194	0.615±0.11
Vd	L	5.218±0.49	47.13±3.35
Half life	h	1.79±0.260	3.158±0.59
MRT	h	2.806±0.34	26.75±1.02
Tmax	h	0.727±0.021	1.05±0.106
Cmax	mg/L	1.503±0.064	12.5±1.232

Particle size

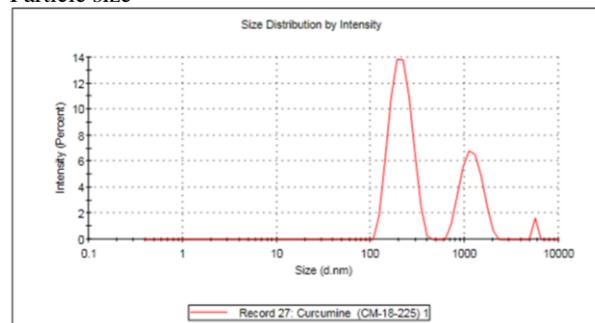


Figure 1: Particle size of nanoparticles.

Zeta Potential

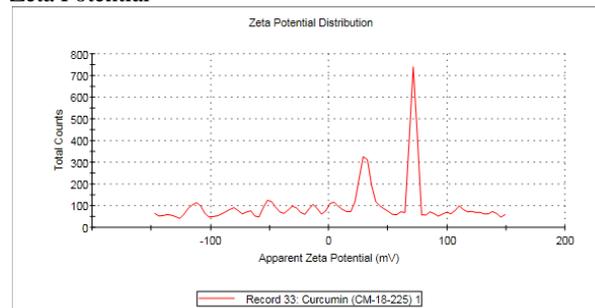


Figure 2: Zeta potential of nanoparticles.

Determination of minimum inhibitory concentration (MIC) of Cur NPs: The MIC of pure curcumin, Cur NPs and 10% DMSO as negative control are illustrated in Figure 3. The results showed that the value of MIC of pure curcumin and Cur NPs were very less as compared to commercially available ampicillin against the tested strains.

In vivo pharmacokinetic study of curcumin in rabbits: *In vivo* pharmacokinetic study of curcumin in rabbits was done by orally administering pure curcumin and curcumin loaded PLGA nanoparticles at a dose rate of 15mg/kg body weight.

The mean blood concentration-time curve of the pure curcumin and curcumin PLGA nanoparticles are shown in Figure 5, demonstrating that the concentration-time curve was best described by the two-compartment model for curcumin. The pharmacokinetic parameters

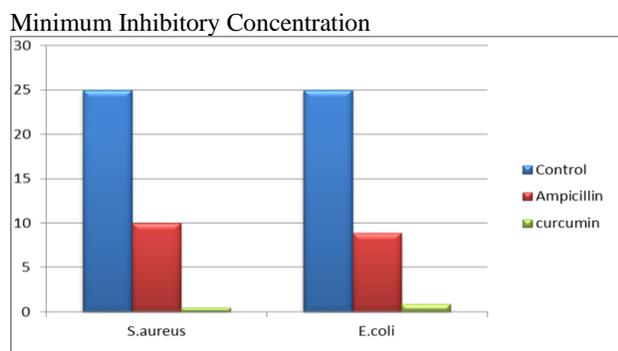


Figure 3: Comparative MIC representations of curcumin against *S.aureus* and *E.coli*.

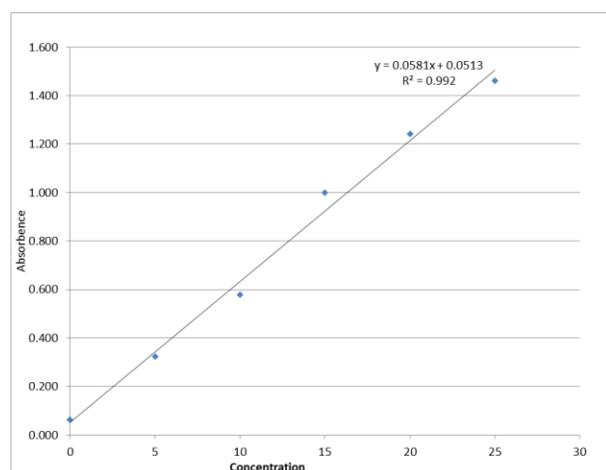


Figure 4: Standard curve of Curcumin for determining concentrations in blood samples.

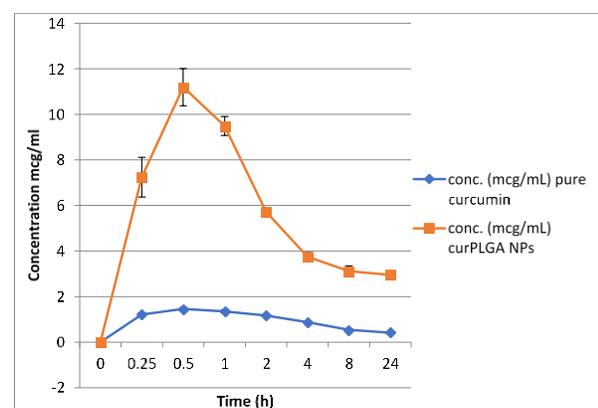


Figure 5: Mean and SEM blood concentration Vs time profile of pure curcumin and CurNPs.

showed that, pure curcumin was absorbed in rabbit, and the C_{max} of curcumin in rabbit blood was $1.5 \pm 0.064 \text{ mg/L}$ and the T_{max} was 0.727 ± 0.021 , and the pharmacokinetic parameters of CurNPs showed that curcumin was absorbed higher in rabbit, with the C_{max}

of $12.5 \pm 1.232 \text{ mg/L}$ and T_{max} of 1.05 ± 0.106 . The values of pharmacokinetic parameters such as area under curve, clearance, volume of distribution, half-life, mean residence time were $13.605 \pm 0.64 \text{ h.mg/L}$, $1.34 \pm 0.194 \text{ L/h}$, $5.218 \pm 0.49 \text{ L}$, $1.79 \pm 0.260 \text{ h}$ and $2.806 \pm 0.34 \text{ mg/L}$ respectively for pure quercetin and were $95.38 \pm 0.89 \text{ h.mg/L}$, $0.615 \pm 0.11 \text{ L/h}$, $47.13 \pm 3.35 \text{ L}$, $3.158 \pm 0.59 \text{ h}$, and $26.75 \pm 1.02 \text{ mg/L}$ respectively for CurNPs. The other pharmacokinetic parameters of CurNPs and pure curcumin were described in Table 1.

Discussion

Formation of nanoparticles depends upon the interaction of PLGA with aqueous phase. The nanoparticle size depends upon the PLGA and curcumin ratio and the stirring speed of magnetic stirrer and the biological performance will vary based upon the size characteristics. The behavior of nanoparticles-based drug delivery systems depends on the physical and chemical properties of the nanoparticles, like entrapment efficiency, zeta potential, polydispersity index and particle size.

Zeta Potential is the charge present on the surface of particle. The storage stability of a colloidal dispersion is affected by zeta potential. Zeta potential describe the electric barriers that hinders the nanoparticles from agglomeration and aggregation. If a particle acquire very low zeta potential it may likely to aggregate because electric repulsion would not be adequate. For a stable nano suspension, the required value of zeta potential should be -30 mV for electrostatically stable suspension and -20 mV for sterically stabilized systems. If a system of nanosuspension has zeta potential has value of 20 mV it shows that nanosuspension is likely to aggregate quickly and has a short-term stability. A value of zetapotential give the information about possible storage conditions and predict the discarding time of nanosuspension. If the zetapotential value varies between -5 to 5 it shows that the suspension will have fast aggregation. (Jacobs *et al.*, 2000) In the present study the zeta potential was found to be -1.35 mV . As the PLGA polymer is uncharged polymer it gives negative zeta potential near to neutrality. Its value is negative because PLGA polymers acquire a slightly negative zeta potential which is likely to be due to the ionization of glycolic acid in PLGA polymer. The negative zeta potential is beneficial in drug delivery and prolonging the circulation time property.

Polydispersity index is defined as the ratio of size difference to mean diameter of particle. It gives information about the homogeneity of particles in a suspension and its range varies between 0-1. If the value of PDI is close to zero it shows that the suspension is homogenous and if the value is greater than 0.3 it shows

the heterogeneity of suspension. High values of PDI show that there is much variation in particle size.

In present study the PDI of curcumin loaded Nano suspension was found to be 0.368 which shows that solution is homogenous. The conditions that can affect the size distribution are the molecular weight, concentration and condition of mixing.

Zeta size is an important parameter for characterization of nanoparticles. The prepared nano formulation exhibits a particle size of 420.5nm which lies within the range of 10-1000nm which shows nanoparticles were formed. An electron microscope is used to access the size and morphology of particle. Size of nanoparticle can affect the rate of release of drug and so also have impact on absorption of drugs. If the size of nanoparticle is small it shows an increase in surface area and ultimately an increase in drug release. (Rabanel *et al*, 2015).

Entrapment efficiency was calculated by drawing the standard curve, utilizing UV-visible spectrophotometric analysis at 402nm wavelength and then by putting values in the given equation. The encapsulation efficiency of the prepared formulation was 89.4%. These results were higher than those of reported studies, like in another study curcumin loaded poly (lactic-co-glycolic acid) (PLGA) nanoparticles have encapsulation efficiency of about 79%. Fewer reports identified that curcumin loaded polylactic acid (PLA) nanoparticles have encapsulation efficiency of around 95%.

Curcumin is the most active constituent of Turmeric that is recognized because of its various medical uses. Despite of all other uses one of the documented use of curcumin is its antibacterial activity. In the present study the anti-bacterial activity of curcumin was assessed against four genera of bacteria's including two-gram positive strains and two-gram negative strains.

Curcumin can intensify the permeability of liposomes because of it is a lipophilic molecule and has amphipathic nature. Curcumin also has membrane disordering properties. It can cause the thinning and disruption of bacterial membrane at high concentration.

In this study, the MIC values of free curcumin and Cur-loaded nanoparticles were evaluated by microdilution technique. In the broth microdilution method, MIC values could not be determined spectrophotometrically due to turbidity formation, which gave absorbance at 570 nm caused by insoluble Cur compounds. The high optical density resulting from the increase in turbidity is considered as bacterial growth in this method; thus, it was detected that the free curcumin gave false positive results by causing visible turbidity due to the insolubility of curcumin. However, this situation was not observed for curcumin-encapsulated PLGA NPs. There is no doubt that NP systems are good solvent systems as alternatives to other organic solvents

such as ethanol, methanol and DMSO (Ravichandran, 2013).

The pharmacokinetics and model parameters were calculated by the practical pharmacokinetic APO software. The pharmacokinetic parameters showed that, after oral administration of pure curcumin the AUC was 13.605 ± 0.64 and when the AUC of curcumin loaded nanoparticles was observed it was found to be 95.38 ± 0.891 which is much greater than simple curcumin. The value of C_{max} which is the peak plasma concentration is also increased in CUR NP. The value of C_{max} for simple curcumin was $1.503 \pm 0.064 \mu\text{g/ml}$ while for curcumin nanoparticles its value is $12.50 \pm 1.232 \mu\text{g/ml}$. The value of T_{max} of pure curcumin was found to be $0.72 \text{ h} \pm 0.021$ and the value of T_{max} for CUR NPs was $1.05 \text{ h} \pm 0.106$ which is significantly greater than pure curcumin. The mean residence time for CUR NP is also increased to $26.75 \text{ h} \pm 1.02$ while its value for pure curcumin was $2.806 \text{ h} \pm 0.34$. The increase in C_{max} and T_{max} values after administering curcumin loaded PLGA nanoparticles can be explained by controlled and sustained release of curcumin from PLGA nanosystem. PLGA prevents the rapid degradation of curcumin in gastrointestinal tract as well, which aids in greater concentration of curcumin in blood. As a result half-life of curcumin increase from $1.79 \text{ h} \pm 0.260$ to $3.158 \text{ h} \pm 0.59$. PLGA nanoparticles also contribute to enhanced and easy penetration of curcumin loaded PLGA nanoparticles across the gastrointestinal membrane.

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