Original Article

Poly Lactic-Co-Glycolic Acid Nano-Carriers for Encapsulation and Controlled Release of Hydrophobic Drug to Enhance the Bioavailability and Antimicrobial Properties

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Attaullah Bukhari, PhD[1], Zuha Fatima, MS[1], Madiha Atta, PhD[2], Arif Nazir, PhD[1], Samar Z. Alshawwa, PhD[3], Hadil F. Alotaibi, PhD[3], and Munawar Iqbal, PhD[4]

Abstract

This study focusses on the fabrication of nano-carriers for delivery of ciprofloxacin through the nanoprecipitation process. This was done to examine the release of drug at the pH of stomach to find out the antibacterial action of ciprofloxacin loaded nanoparticles (NPs). Prepared NPs were characterized by Fourier Transform Infra-Red (FTIR) spectroscopy, Scanning Electron Microscopy (SEM), and particle size analyzer (PSA) techniques. Drug yield, loading, and sustained release was studied as function of time (up to 8 h). Antibacterial activity of ciprofloxacin loaded NPs were also determined against different gram-positive and gram-negative bacteria. Results revealed that nanoprecipitation is a suitable method for encapsulation of ciprofloxacin in poly(lactic-co-glycolic acid) PLGA NPs. The drug yield and drug loading were found to be 60%. The size range of NPs observed by PSA was in the range of 5.03–6.60 nm. It can be concluded that nanoformulation of ciprofloxacin loaded PLGA NPs can be used in stomach for longer period of time to enhance the bioavailability of the drug.

Keywords

nanoparticles, nano-carriers, ciprofloxacin, drug release, nanoprecipitation, antibacterial properties

Introduction

The drug dosage is quite a serious problem now a day. The dosage has to be given time and again to ensure the patient compliance. Due to the rapid growth in clinical medicines, the desire to have a control on effective delivery is a necessity. The need for polymeric drug delivery was driven by the non-specific distribution of drugs, toxicity, and response of the body to the certain drugs.[1-4] The biodegradable sutures were first obtained by hair, tendons, plant, and wool fibers by Egyptians. The earliest synthesized polymers were used in the 1960s and 1970s. The biodegradable materials in that time used were poly lactic-co-glycolic acid (PLGA), polyglycolic acid (PGA), and polylactic acid (PLA). Biodegradable polymers used in drug delivery have more importance because of the nature of degradability in body and their biocompatibility. These polymers are biocompatible due to their breakage in the body and producing non-toxic end-products in terms of carbon dioxide and water.[5-7] The hydrolysis of PLGA and PLA results in lactic acid, glycolic acid, and for this reason, they have little or no toxicity.[8,9]

Nanoprecipitation is a simple and quite rapid technique. The nanoparticles formation is instantaneous and quick. Briefly, two solvents of different nature must be immiscible. Ideally, the organic solvent must be capable to dissolve both

[1] Department of Chemistry, The University of Lahore, Lahore, Pakistan
[2] Department of Biochemistry, The University of Lahore, Lahore, Pakistan
[3] Department of Pharmaceutical Sciences, College of Pharmacy, Princess Nourah bint Abdulrahman University, P.O. Box 84428, Riyadh 11671, Saudi Arabia
[4] Department of Chemistry, Division of Science and Technology, University of Education, Lahore, Pakistan

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Corresponding Author:
Munawar Iqbal, Department of Chemistry, Division of Science and Technology, University of Education, Lahore 54770, Pakistan.
Email: bosalvee@yahoo.com

drug and polymer but these must not be dissolving in non-solvent systems.[10] This approach includes the nano-precipitation of an organic polymer mixed in an organic solvent via stirring and diffusion in the presence of a surfactant.[11,12] As soon as the organic solvent containing drug and polymer diffuses, the drug is entrapped. The rapid synthesis of nanoparticles occurs due to the interfacial attractions and turbulences between two solutions; one is solvent and the other is non-solvent. These interfacial turbulences are governed by shear rate, diffusion, and surface tension variations. The solvent used in this technique is miscible in aqueous phase, and therefore, it is applicable to lipophilic drugs.[13,14] Nanoprecipitation does not require very high temperatures, high sonication rates, shearing, and stirring speeds. Besides surfactants, toxic organic solvents are also avoidable. Consequently, a very low portion of the drug is released outside the medium with maximum entrapment efficiency.[15-18]

Sustained drug delivery has given the better approach in comparison with conventional dosage forms.[19] Controlled release of drug is better than conventional methods because it provides more therapeutic outcomes and is used for number of drugs either hydrophobic or hydrophilic.[20] The drug ciprofloxacin belongs to the class of quinolones. It is effective against a number of bacterial infections mostly gram-negative like *pseudomonas aeruginosa.* It is hydrophobic in nature; it has short half-life and it has low bioavailability in the body. It has half-life of 3.5 h. Due to its less retention time in the body, it is needed more strongly to have ciprofloxacin preparation for controlled release and prolonged drug release. Ciprofloxacin entrapped in degradable nanoparticles prolongs the safe release of drug and promote bioavailability of drug.[21] Recently, drug delivery with nanoparticles has shown the enhancement in the delivery of drug at respective target. The commercial antibacterial medicines have poor solubility in water and also the market available tablets have short half-life prior to this concept of drug loading. Most of the research done in this field have nanoparticles loaded with drugs, have poor bioavailability, and burst drug release effect.[22,23] In the present study, the focus is kept on minimal burst release effect of ciprofloxacin and PLGA to improve the half-life of drug ciprofloxacin in joining with PLGA.

The main purpose of this study was to fabricate nanocarriers for drug delivery using PLGA (50;50) employing nanoprecipitation process, to encapsulate the hydrophobic ciprofloxacin in PLGA nanoparticles. A simple process to develop and optimize controlled release of drug ciprofloxacin to form NPs delivery systems with the aim to improve the permeability of ciprofloxacin using standard polymer combination and surfactants. This is done to observe the release pattern of ciprofloxacin from drug loaded NPs at pH 7.4 and to check the antibacterial effect of fabricated ciprofloxacin loaded NPs.

Material and Methods

The analytical grade reagents and chemicals were purchased from Sigma Aldrich. The PLGA NPs were prepared by a precipitation-solvent evaporation method with some modification to that offered by Fessi et al.[10] Polymer molecular weight is 30–60 kDa with 1:1 ratio. Organic phase was prepared by taking 30 mg polymer PLGA. This polymer was mixed in 5 mL ethyl acetate in a conical flask. Drug was not added into this solution. The mixture was constantly mixed with magnetic stirring until it was added into the aqueous phase.

Solvents are generally selected based on drug solubility. If a drug is hydrophobic in nature and has low solubility, then solvents like ethyl acetate, acetone, acetonitrile, and ethanol are used. Solvents used in nanoprecipitation techniques must be miscible with water, because these solvents facilitate diffusion at higher level and thus helps in nanoparticles formation of smaller size. Acetone and ethyl acetate have high diffusion rates and therefore they favor the small NPs size, and also nanoparticles formulated in these solvents have narrower distribution. The solvents with low diffusion rates result in bigger nanoparticle sizes such as tetrahydrofuran forms NPs of large size and broad distributions.[24] Aqueous phase was prepared by adding .1 g of PVA into 10 mL water. 1% w/v solution of PVA was prepared. 5 mg ciprofloxacin was also added into the aqueous phase. The solution was kept on constant stirring for half an hour at 80 rpm.

During the preparation of NPs, polymer and drug should not be soluble in the anti-solvent to avoid anti-diffusion of drug. Organic phase to aqueous phase ratio is reported in many researches [89], and if the aqueous phase proportion increases with respect to solvent which is organic phase, it produces particles with smaller sizes. Generally, a concentration range between .1–1% to 7% w/v of stabilizer is sufficient for stabilization of the nanoparticles.[25,26]

Preparation of PLGA NPs

The organic phase was added gradually 1 mL/min into aqueous phase under constant stirring at 50 rpm. It was done with injection syringe located in the middle. At this rate, syringe must be located into the center of aqueous phase. The speed of stirrer was kept at 50–100 rpm to make sure of effective stirring. Organic phase was removed from solution by evaporating it overnight.[27] Nanoparticles were recovered by ultracentrifugation for 10 min at 13,000 rpm, washed twice with water to remove any unentrapped drug. The samples were kept in freeze dryer for 24h at −5°C. The washed samples were kept in desiccator for minimum 3 days.

Drug Release analysis of Loaded PLGA NPs

Stock solution was prepared by weighing a specific amount of solid ciprofloxacin and was dissolved into appropriate amount

of liquid. So 5 mg of drug ciprofloxacin was taken and dissolved into 1 mL of phosphate buffer saline (PBS). This mixture was placed on vortex mixer for an hour and dilutions were made. The molarity of stock solution was found to be .015 M or 15 mM. 100 µL of volume is taken from 15 mM solution of pure ciprofloxacin and 900 µL of phosphate buffer saline was added and the dilution was made. The concentrations of the dilutions were 1.5 mM, 1.2 mM, .9 mM, .6 mM, and .3 mM, respectively. 4.5 mg/µL of nanoformulation was dissolved through vortex mixer. NPs solid homogeneous dispersion was mixed into 1 mL of phosphate buffer saline.

A Franz cell apparatus was taken. A dialysis membrane that separated both compartments was placed on the chamber. An accurately weighed quantity of 4.5 mg of PLGA ciprofloxacin NPs was placed on the membrane. The membrane was used to separate the upper compartment from the chamber. One mL of pH 7.4 PBS was placed on the membrane. The receptor chamber which is in lower chamber was completely filled with 10 mL buffer. The buffer present in lower compartment was wetting the membrane and nanoparticles. To avoid the evaporation, the acrylic top was tightly sealed. At fixed time intervals, samples were subjected to the analysis of .5 mL and replenish it with freshly prepared buffer. Dissolution studies were done at 37°C and 80 rpm.[28] To determine the content of ciprofloxacin in nanoparticle formulation, 3.35 mg of nanoformulation which was prepared was taken and dissolved into 2 mL of DCM and then 4 mL of water was added. The solution was kept on magnetic stirrer for 7–8 h and 1 mL of this solution was withdrawn to check the drug contents, which was monitored at 278 nm in UV Spectrophotometer (Schimadzu 1601).[29] The absorbance noted was .622 nm. Drug efficiency was calculated by following formula:[30]

$$\text{Drug loading efficiency}\ (\%) = \frac{\text{Drug weight in NPs}}{\text{Total weight of NPs}} \times 100 \quad (1)$$

Characterization of Ciprofloxacin Loaded PLGA NPs

Prepared nanoparticles were characterized using scanning electron microscope, particle size analyzer, and FTIR spectroscopy. FTIR of ciprofloxacin nanoparticles with PVA, pure ciprofloxacin, polymer, and nanoparticles were subjected to analysis. FTIR spectra were obtained using a PerkinElmer FTIR spectrometer. The IR spectra was obtained in the spectral region of 450–4000 cm^{-1}. Ciprofloxacin loaded nanoparticles were subjected to analysis by the Nova SEM. The particle size and distributions were measured by BT-90 nano laser particle size analyzer at 25°C and scattering laser angle was 90°. A 1 mg nanoformulation was mixed well in 1 mL water and shaken well until the homogeneous solution is formed.

Antimicrobial Studies

2 g agar and .8 g of nutrient broth were added to 100 mL distilled water and autoclaved in an Erlenmeyer flask. Cotton plug was applied to the opening of flask and was sterilized in an autoclave for 15 min at 121°C. Petri plates were sterilized in hot oven to avoid contamination. 15–20 mL of agar was poured into the petri plates, which was then solidified and inoculated with gram-negative and gram-positive bacteria like *pseudomonas aeruginosa.* Wells were formed in the petri dishes of .4 cm. 20uL of sample suspension was added into the walls. 3.5 mg of sample suspension was made into 1 mL of water. After 24 h, zone of inhibition was observed at 37°C. For this antibacterial activity, different bacterial strains are used including *E. coli, P. aeruginosa, S. aureus*, *K. pneumonia, B. subtilis*, and *B. cereus* species.

Results and Discussion

Properties of Ciprofloxacin Loaded PLGA NPs

Organic phase containing PLGA dissolved into ethyl acetate was injected into aqueous phase. Aqueous phase contains PVA as a surfactant to avoid aggregation of nanoparticles. Organic phase was injected into aqueous phase via syringe. The nozzle or lip of syringe was kept dip into the aqueous phase. As the organic phase was added, the colorless solution turned milky white. That confirmed the nanoprecipitation of polymer. Nanoparticles were made simultaneously as soon as organic phase was added into the aqueous phase at constant magnetic stirring. Once the nanoprecipitation was confirmed, sample was centrifuged at 13,000 rpm. Nanoparticles were recovered; further NPs were freeze dried for 24h at −5°C. Prepared nanoparticles were characterized using scanning electron microscope, particle size analyzer, and FTIR.

SEM results in Figure 1 have shown the morphology of ciprofloxacin loaded PLGA nanoparticles. PVA used as a surfactant in the nanoprecipitation method minimizes the interfacial tension as the organic solvent is added into the aqueous phase. Surfactant prevents the aggregation of NPs. The small nanoparticle size was attainable due to the constant stirring rate. Constant stirring during the addition of organic phase into aqueous phase affects the nanoparticle size and its morphology. The NPs in the image have small particle size. The addition of PVA into the aqueous phase provided the protection against aggregation. SEM studies confirmed the presence of polymer in which drug was entrapped. In Figure 1 of SEM image, the size range is found to be 200 nm. The particle size observed by SEM is relatively larger than that by particle size analyzer (PSA). The size of nanoparticles depends upon the amount of surfactant used. Polymer concentration also affects the size of nanoparticles. The nanoparticles having smaller size can be used in sustained delivery of drug. It was observed that nanoparticles were spherical in shape and were in cluster form. SEM images and particle size analyzer proved that nanoparticles were in nano size.

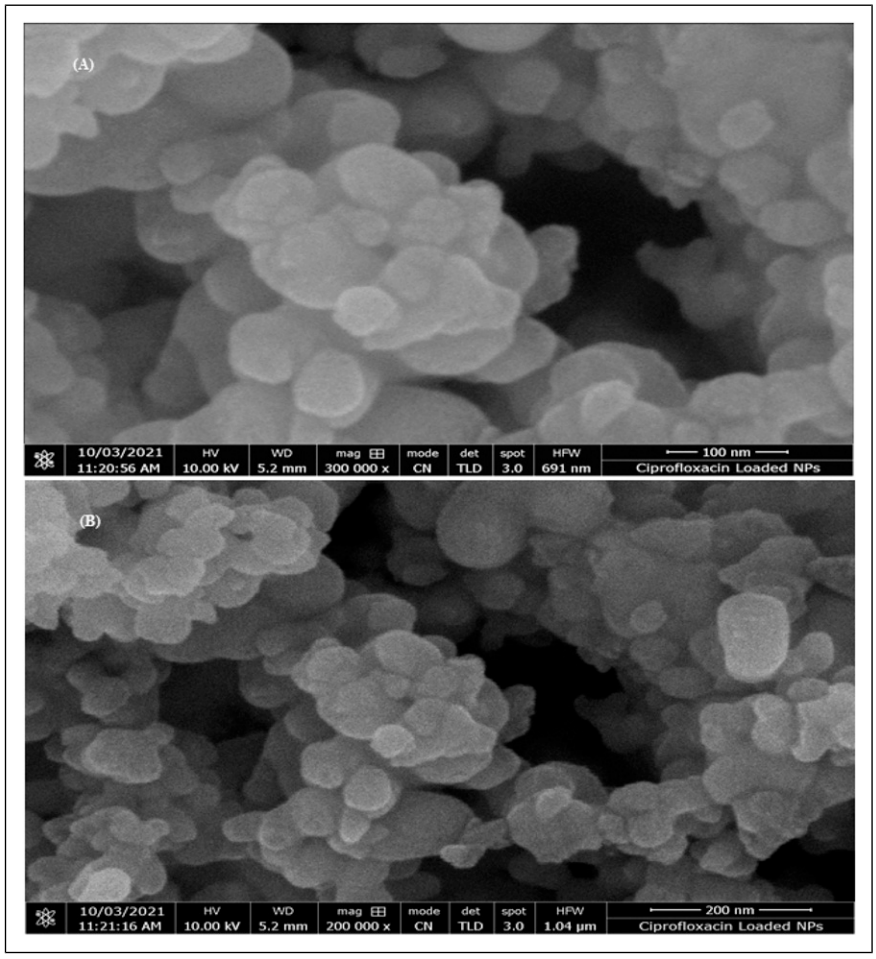
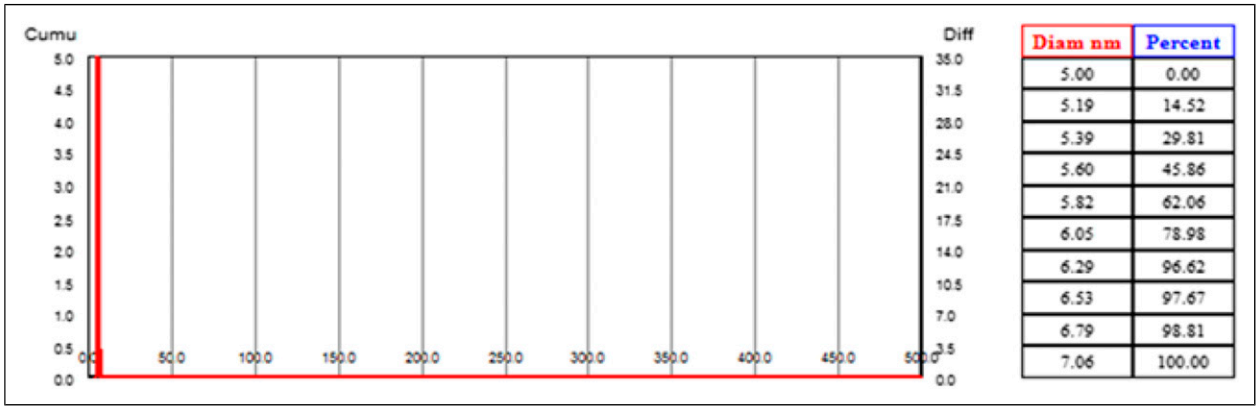


Figure 1. Scanning electron microscopy image of CIP-loaded poly lactic-co-glycolic acid nanoparticles showing the morphology of synthesized material.



Diam nm	Percent
5.00	0.00
5.19	14.52
5.39	29.81
5.60	45.86
5.82	62.06
6.05	78.98
6.29	96.62
6.53	97.67
6.79	98.81
7.06	100.00

Figure 2. Particle size analysis of poly lactic-co-glycolic acid nanoparticles endorsing the size in nanometer scale.

Particle size was analyzed with particle size analyzer and its report is shown in Figure 2. The poly-dispersity and the mean particle size of ciprofloxacin drug loaded NPs are also shown. It was observed with laser scattering beam, the mean particle size was in the range of 5.03–6.60 nm with a narrow size distribution of nanoformulation. For ciprofloxacin NPS, the poly-dispersity index was .011. D10 means 10% particles in the liquid are smaller than 5.13 nm. D90 means 90% particles are smaller in size than 6.19 nm. Similarly, for D25, 25% particles are smaller than the 5.23 nm in size. D10, D50, and

D90 give the total concept and idea of total size distribution.[31] The size distribution and mean size of particles are important factors and parameters for the manufacturing of physical and chemical stable nanoparticle drug delivery careers. It can be stated from Figure 2 that the size range of ciprofloxacin loaded NPs were observed within the range of 5.03 to 6.60 nm range. The poly-dispersity index was also found to be .011 for the loaded NPs. It is observed by the histogram that nanoformulation showed mono-dispersity in particle size distribution.

FTIR Analysis

It is documented that drug release highly depends upon physical and chemical nature of the compound that is loaded or encapsulated in the polymer. It also depends upon how the compound interacts with polymeric matrix and how it forms the hydrogen bonds. Ciprofloxacin has carbonyl group (=C), hydroxyl group (OH), and amino groups (NH) which could possibly interact with hydroxyl and carbonyl group of PLGA.[27] The FTIR spectrum of PVA is shown in Figure 3. The first peak of PVA observed at 3380 cm^{-1} is due to the presence of –OH bond stretching. This peak is mostly broader than other most peaks. This major peak is due to the presence of –OH group which arises from molecules of water. This explains the presence of water molecules adsorbed on the surface. The second peak is present at 2928 cm^{-1} due to $–CH_2$ stretching vibrations. The peaks at 1730–1690 cm^{-1} are due to the presence of –C = O carbonyl bond stretching vibrations

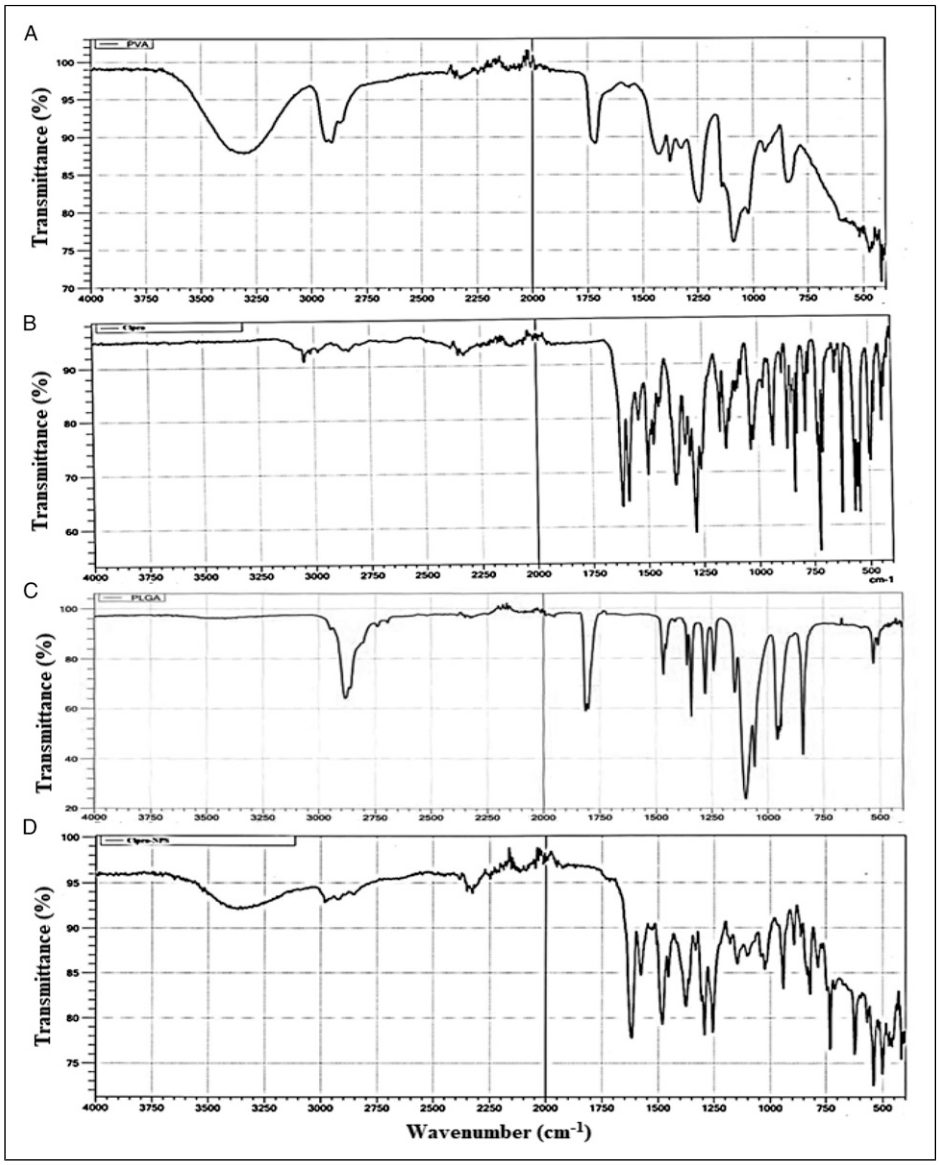


Figure 3. Fourier transform infra-red spectra of (A) PVA, (B) pure ciprofloxacin, (C) poly lactic-co-glycolic acid, and (D) ciprofloxacin loaded poly lactic-co-glycolic acid nanoparticles indicating the presence of various functional groups.

and –CO from acetate group also shows peak in this region. The peaks at 1450 cm^{-1} are due to the presence of $–CH_2$ group and that's why it has –C-H bending vibrations. The peak at 1250 cm^{-1} is due to the deformation vibration of –C-H bond. The peak at 1095 cm^{-1} is present because of –C-O stretching of acetyl group and peak at 870 cm^{-1} is observed due to the presence of –C-C stretching.[27] The first sharp peak at 2817 cm^{-1} is due to the presence of symmetrical stretching of –C-H bond. The second sharp peak appeared at 1470 cm^{-1} due to the presence of –C-C bond antisymmetric deformation. The peak from 1290 cm^{-1} to 1120 cm^{-1} is due to the presence of –C-O symmetric and antisymmetric stretching of –C = O. The bands at 3500 cm to 3000 cm show the presence of –OH group of PLGA. The intense peaks at the range of 1750 cm^{-1} to 1650 cm^{-1} attribute to the stretching of the carbonyl groups in the two monomers. The IR spectra of chemicals help to understand the chemical composition and bonding arrangement of monomers and carbon chain.[28] The infra-red spectra of pure ciprofloxacin show the band at 3050 cm^{-1} due to the stretching of –CH stretching vibration and the –OH bond.[13] The intense peak at 1750 cm^{-1} to 1700 cm^{-1} is because of the stretching of –C = O bond and it is modified by the substituents. The peaks in this range are due to the presence of cyclic five membered rings which contain –C = O stretching. The peaks from 1600 to 1650 cm^{-1} are indicative of aromatic and aliphatic amines of the quinolone so the –N-H bond in this region is deformed. The peak at 1500 cm^{-1} is due to presence of –O-C-O of carboxylic acid. The peak at 1400 cm^{-1} is showing the presence of –OH in plane deformation, the peaks at 1350 cm^{-1} and 1270 cm^{-1} are present because of –C-N stretching and do to the presence of –OH group in this region. The strong peak present at 1160 cm^{-1} is indicative of both –C-C and –C-O stretching in the amorphous phase. The peaks in the region of 1050 cm^{-1} to 1100 cm^{-1} are present because of fluoro group stretching which is attached to the side of the ring and series of bands shows the presence of fluoro group. The peaks which are in the region of 1000 cm^{-1} to 750 cm^{-1} are present due to the alkene CH out of plane deformation. These are very useful bands to understand the pattern of substitution. The bands present between 750–500 cm^{-1} are due to the –CH deformation and the broad band at 1250 cm proves the deformation of –CH band at 680 cm^{-1}.[30] The most prominent band present at 1750 cm^{-1} to 1700 cm^{-1} corresponds to the presence of carbonyl group.[32]

From the chemical structure of PLGA, bands found in the FTIR spectrum were relative due to the presence of carbonyl group and ester bonds. Stretching vibrations were observed at 2890 cm^{-1} due to the presence of –C-H bond in the region due to the presence of alkanes in PLGA. While the bending vibrations were observed in the spectra at 1280 cm^{-1}. Symmetrical and asymmetrical stretching of $–CH_2$ and $–CH_3$ is happening in this region of 2850 to 2920 cm^{-1}. The intense peak was observed at 1780 cm^{-1} due to the fact of carbonyl group present in two monomers of PLGA. The bands present in 1100 to 1180 cm^{-1} are found due to the presence of aliphatic polyesters stretching of –C-O group. The sharp peak at the region of 1350 cm^{-1} attributes the antisymmetric and symmetric stretching of aldehyde group and –C-O present in the PLGA polymer.

The band present at 1467 cm^{-1} is due to the presence of –C-H bending of alkane. The band in the region of 1250 to 1470 cm^{-1} is bending of –OH group of carboxylic acid. The band at 1310–1250 cm^{-1} is due to the presence of –C-O stretching of ester group. The bending of –CH is happening in the region of 800–900 cm^{-1}. The broad spectrum in the range of 3000 to 3500 cm^{-1} is indicative of hydroxyl group present in the region. Ciprofloxacin loaded PLGA NPs showed a broad peak in the region of 3000–3500 cm^{-1} which is missing in the ciprofloxacin spectra. This peak shows the presence of –OH group due to hydrogen bonding between water molecules. The spectrum was quite broad in the IR spectra of PVA as the –OH group is water and it is adsorbed on the surface. The quite narrow spectra of –OH group is indicative of the fact that water is not adsorbed on the surface of nanoparticles, and hence, drug release showed a slower burst release. The peak at 3000 cm^{-1} is indicative of the presence of $–CH_2$ group. Band at 2300 cm^{-1} is indicative of the presence of –CN stretching vibrations. The sharp peak observed at 1650 cm^{-1} showed the presence of –C = O bond which is present in both ciprofloxacin and PLGA. This functional group is present in ciprofloxacin, PVA, and in PLGA too. PLGA gives a sharp peak in this region because of the multiple functional groups present in one single monomer. The band from 1500 to 1600 cm^{-1} is due to ciprofloxacin quinolone ring. The peak at 1500 cm^{-1} is due to the presence of carboxylic acid O-C-O which is same as present in ciprofloxacin. The peak range from 1250–1450 cm^{-1} is the indicative of –OH stretching and –C-N stretching, respectively. The peak present at 1200 cm^{-1} is due to the stretching of both –CH and –CO stretching. The strong peak at 900 cm^{-1} is due to the alkene C = C stretching. The peaks at 800–750 cm^{-1} are due to the CH out of plane deformation. The ciprofloxacin loaded PLGA nanoparticles shares the quite same spectra of pure lipophilic ciprofloxacin which quite proves that ciprofloxacin is entrapped inside the polymer core.

Nanoparticles Yield

The yield of nanoparticles was observed to be around 60%. The total amount of polymer used was 30 mg with the 5 mg drug added. The obtained nanoparticle powder was 21 mg in weight.

$$\text{Nanoparticles yield}\,(\%) = \frac{(\text{weight of nanoparticles})}{(\text{weight of polymer and drug fed initially})} \times 100 \quad (2)$$

Percentage yield increases with the increase in polymer concentration. As in most cases, if the amount of polymer is increased, the yield (%) will also increase.

Dissolution Studies of Ciprofloxacin Loaded PLGA NPs

Studies have been trying to overcome the less bioavailability of orally administered drugs that were in use for years. So this study is meant to observe the bioavailability of ciprofloxacin loaded NPs at pH 7.4. The stock solution was prepared by accurately measuring 5 mg of pure ciprofloxacin and dissolved into the 1 mL of PBS. Total 5 dilutions were made of different concentrations. The concentrations of the dilutions were 1.5 mM, 1.2 mM, .9 mM, .6 mM, and .3 mM, respectively. The stock solution was subjected to a UV spectrophotometer at 278 nm.[29] The concentration of the unknown samples was found out. Straight-line graph was obtained. This calibration curve is prepared to find out the control drug release of ciprofloxacin and by drawing the standard curve cumulative drug release was observed. The graph is drawn between absorbance and concentration. 4.5 mg of nanoparticles were taken and accurately measured on weighing balance. The accurately measured nanosuspension was added into the 1 mL of phosphate buffer saline and mixed on vortex mixer. It was mixed until the nanosuspension was homogeneously mixed up. The nanosuspension was poured on the upper compartment of Franz diffusion cell and a membrane was separating both compartments. In the lower compartment, a 10 mL of phosphate buffer saline was poured. After every one hour, to analyze the sustained release of drug, .5 mL of PBS was taken from the lower compartment through a syringe and the upper compartment was replenished with new PBS. The extracted .5 mL of PBS was subjected to UV spectroscopy and absorbance was measured for 8 h.

Drug release was observed for 8h with 1-hour interval of drug sustained release in the phosphate buffer saline solution. Figure 4 displays an initial slower burst release of loaded drug in the first 5 h of incubation. The data demonstrates that part of the drug was well covered or protected at the initial stages of drug release. Somehow, the burst release effect here shows that some of the drugs were not loaded as well as the drug which was present near to the surface. In the last hours, a slow and continuous release of drug release has been observed. It has been observed that primarily, drug was released in small amount and burst phase was also observed after 3–4 h. The drug was noted to be released 82% after 8 h. The release contains two steps: the one is burst release and the other one is steady one. The burst effect happens because of the unbound particles of the drug adsorbed on the surface and near-surface and attributed by the factor of the strong concentration gradient between two mediums. After the burst release effect, the drug is delivered to the body linearly and slowly because diffusion from the inner core of the particle makes it diffuse slowly. The modified method of nanoprecipitation is better for hydrophobic drugs for the encapsulation and release of drugs. It helps to deliver the NPs as delivery carriers. The release is not complete which attributes to the fact that drug and polymer have strong interaction. In this drug release profile, the burst effect is minimal. It shows that there was minimal drug adherence on the surface as the drug adheres on the top of nanocapsules or sphere. It gives a false alarm of burst release effect which is not controllable if the drug has already adhered to the surface. Less adherence of the drug on the surface of the particle minimizes the chances of burst release, which attributes to more controlled and sustained release of the drug. Cumulative percentage drug release is also affected by the type and quantity of polymer used. The non-toxicity and non-irritant behavior of PLGA makes it one of the best polymers for use in drug delivery. The very minimal lag phase and burst release indicate that the solution had homogeneous drug distribution.

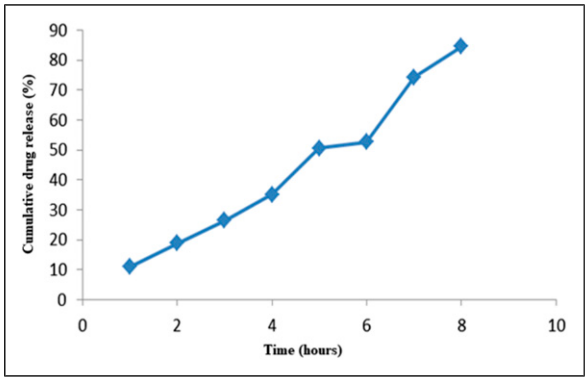


Figure 4. Graphical representation showing the cumulative drug release (%) of CIP-loaded in poly lactic-co-glycolic acid nanoparticles.

Drug Loaded Efficiency of PLGA NPs

The stock solution was prepared of different dilutions. 5 mg of pure ciprofloxacin was taken and dissolved into 1 mL of water. The dilutions were made of different concentrations and absorbance was observed at 278 nm. The nanoformulation which were prepared was taken and 3 mg was dissolved into 2 mL of DCM and 4 mL of water was also added into the above mentioned solution. The nanosuspension was kept on magnetic stirrer for 6–7 h. The drug loading found was 59.9%.

Antimicrobial Activity

Antimicrobial studies were also observed against ciprofloxacin loaded PLGA nanoparticles and market available ciprofloxacin tablet. In this method, standard conditions were applied to study the inhibition of bacterial growth. For this test, culture medium was prepared according to the requirements of different gram-positive and gram-negative bacteria. Slants were prepared and freshly prepared bacteria were aseptically inoculated. After the prescribed time was given, zone of inhibitions was calculated for prepared nanoparticles formulation and ciprofloxacin which was bought from the market. Enhanced microbial activity was observed with ciprofloxacin PLGA NPs. Diagrams have shown that market

available ciprofloxacin has less growth or zone of inhibition. 25 µg/mL samples were taken, and by diffusion well method, the solution was poured. The market available tablet had not killed bacteria as compared to the nanoformulation of ciprofloxacin. The zone of inhibition (ZOI) for bacterial gram-negative strain of *Escherichia coli* showed the enhanced bacterial activity as compared to the tablet bought from the market (Figure 5; Table 1). The medium used for the bacterial growth was agar. Ciprofloxacin nanoformulation showed the enhanced activity with zone of inhibition 35 mm while the tablet had the ZOI of 29 mm. The zone of inhibition for *Pseudomonas aeruginosa* was observed about 25 mm. The zone of inhibition of tablet ciprofloxacin was observed 19 mm. *Staphylococcus aureus* is a gram-positive bacterium which was grown on the agar medium to check the ZOI of both ciprofloxacin nanoformulation and for market available ciprofloxacin. The ZOI of nanoformulation and the tablet was 18 mm and 13 mm, respectively. *Bacillus subtilis* is also a gram-positive bacterium which was grown on agar medium and its zone of inhibition with ciprofloxacin nanoformulation was observed 18 mm, and with the tablet, it was noted about 10 mm. *Klebsiella pneumoniae* is a gram-positive bacterium and agar as a medium was used. It showed the better results with ciprofloxacin NPs amongst all bacteria. It zone of inhibition was observed for nanoformulation 11 mm, and for tablet, it was observed 2 mm.

Orally administered drug delivery systems are the best delivery systems among others. It is convenient for patient and it does not cause pain to patient. Orally administered tablets are easy to carry, safe, and easy to take. They are cheap and there is also no need of sterilization. There are two kinds of dose methods in oral drug delivery systems: immediate release (IR) and modified release (MR). The immediate release (IR) means that there is no control or very little control over release

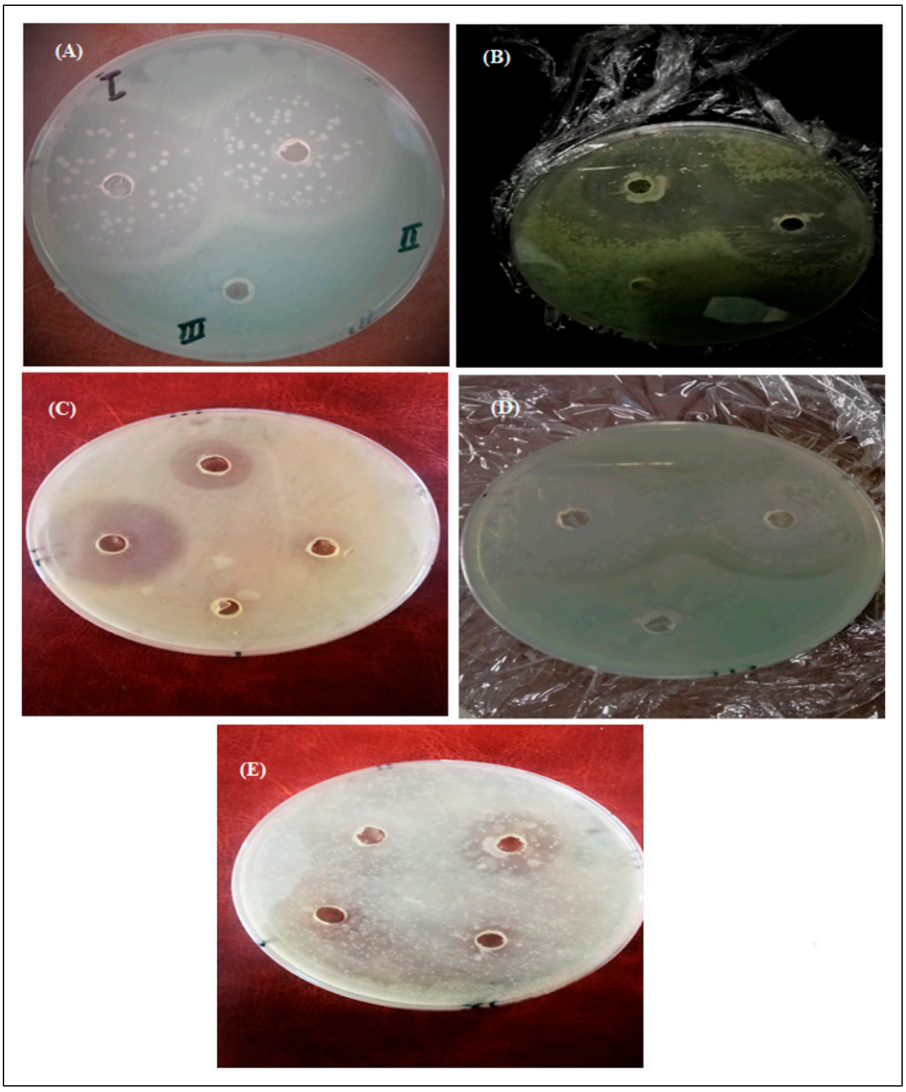


Figure 5. Antibacterial activity of poly lactic-co-glycolic acid nanoparticles showing zone of inhibition against (A) *Escherichia coli,* (B) *Pseudomonas aeruginosa,* (C) *staphylococus aureus,* (D) *Bacillus cerus*, and (E) *Klebsiella pneumoniae*.

Table 1. Comparative Analysis of Antimicrobial Activity of Cipro-PLGA NPs and Market Available Drug Against Different Bacterial Strains.

Microbial strain used	Medium used	Cipro-PLGA NPs ZOI (mm)	Market available Ciprofloxacin ZOI (mm)	Dose applied (mg/mL)	Conclusion
E. coli	Agar	35	29	4	Enhanced activity
P. aeruginosa		25	19		Enhanced activity
S. aureus		18	13		Enhanced activity
B. subtilis		18	10		Enhanced activity
B. cereus		27	24		Enhanced activity
K. pneumoniae		11	2		Enhanced activity

E. coli: Escherichia coli; P. aeruginosa: Pseudomonas aeruginosa; S. aureus: Staphylococcus aureus; B. subtilis: Bacillus subtilis; B. cereus: Bacillus cereus; K. pneumonia: Klebsiella pneumoniae.

rate of drug which is unpredictable and continuously changing the concentration of therapeutic plasma. Modified release (MR) is the advanced medicine oral dosage form for which the drugs release site and time is chosen to achieve the objectives and therapeutic advancements which are usually not presented by conventional dosage forms. Drug release either can be delayed or extended both of which are the types of modified drug release.[33,34]

Drug molecules which are introduced in the body are often degraded by body enzymes. So, to provide them protection from enzymes, carrier systems are manufactured to save drug molecules against degradation. Nanoparticles have high surface area and good solubility in the blood stream, that is why they have better bioavailability. If the particle which is loaded by drug is detected by the body immune system, it might be ejected from the body through urine so this issue can be solved by changing the surface properties of particle. The incorporation of polymer is considered good with drugs because it prevents the drug clearance through the body.[35] They can be used for making of both targeted and localized delivery of drugs. Nanoparticles can be manufactured either by using natural polymers or synthetic polymers.[36,37]

Synthetic polymers have benefits over natural polymers. Synthetic polymers include PGA, PLA, polyanhydrides, polyester amides, polyphosphates, and polycaprolactones.[38] PLGA which is co-polymer of PLA and PGA has a carbon backbone with ester linkages. Aliphatic polyesters involve the PLA, PGA, and PLGA. These are biopolymers and so breakable via hydrolysis of ester bonds. PLGA polymer is biodegradable and biocompatible and therefore less toxic and used in most of the drug delivery systems.[39] Bioavailability of hydrophobic drug is more than that of hydrophilic drug because it has strong interaction with polymer and does not have the affinity towards water. Ciprofloxacin is hydrophobic drug and belongs to a class of flouro quinolones. Ciprofloxacin does not dissolve in water and that is why it is poorly soluble in organic solvent such as methylene chloride, chloroform, dimethyl sulfoxide, and acetonitrile.[40]

Macromolecules to micro polymeric drug delivery systems were progressed by newer methodologies that gave uniformly sized nanoparticles and stable drug deliveries. A number of methods are offered for the entrapment of drug into different polymers to form NPs which completely depends upon the drug and polymer properties. The minimization or variations in solution are done by altering the concentration of drug, pH, non-solvent, or aqueous phase. The particle size is directly affected by the super saturation rate. This step stops right after the concentration of solute decreases below the critical super saturation and nuclei stops growing by coagulation or condensation of solute molecules. This step takes place in two ways; it is the addition of solute molecules to the surface one by one. Either it gets adsorbed on the surface of nuclei or gets diffused from bulk to the solution boundary layer.[41] Condensation stops when the equilibrium rate is achieved by solute molecules and solute concentration decreases. As the rate of coagulation starts increasing, the condensation decreases. It is the joining or adhesion of molecules to each other. It happens when the attractive forces are stronger than the repulsive forces. It happens due to the collision theory, which depends highly on particle size, movement, and particle concentration. The collision frequency leads to coagulation and it also depends on the ratios of attractive and repulsive forces. Stabilizing agents are used for this purpose to prevent coagulation.[41,42]

Flouro quinolones are the drugs ending with the flex flux acid that they work by inhibiting the enzyme DNA topoisomerase 4 + 2 which is called gyrase. It is the enzyme which is responsible for decreasing the tension between super coiled DNA. It interferes with DNA gyrase which is involved in coiling, de-coiling, and

replication.[43] The half-life of ciprofloxacin serum is moderately 4–4.5h with 50–70% of drug excreted by urine from the body as an unmetabolized drug. 10% of drug is excreted by the urine as metabolites. After 24 h, the drug is completely vanished from the system. The extended release of oral tablets can extend the bioavailability of drug in the body by giving one tablet once a day by slowly releasing the drug in the system.[30] Overdosage or underdosage of drug can lead to reverse effects, that is why a moderate drug delivery system is needed to be developed to overcome the problems of bioavailability.[44] PLGA NPs have been used in the transfer of proteins, drugs, and RNAs. It has also rendered an revolutionary part in the delivery of drug for cancer treatments. Researchers have also observed the efficient delivery of vitamins in the body by PLGA nanoparticles and macroparticles.[26]

Many studies have reported the enhanced drug delivery with ciprofloxacin and other drugs encapsulated in PLGA nanoparticles. The purpose of this study is to prepare floating micro-particles of ciprofloxacin by using non-aqueous solvent evaporation method to ensure increased bioavailability and clinical effectiveness of the ciprofloxacin drug by increasing its time in stomach. Low density polymers were used for the making of floating micro-particles by using ethyl cellulose and hydroxy propyl methylcellulose. The fabricated micro-particles were subjected to different analysis such as drug content, percentage yield, and in vitro drug release studies. Results indicated that this technique is an appropriate method for the preparation of floating micro-particles as good yield was obtained with a good spherical morphology. The percentage yield obtained was 65%–85%. Different polymer to drug ratios played a vital role in overall formulation of micro-particles.[35] The findings revealed that PLGA NPs are highly efficient for ciprofloxacin carrier and release to the targeted delivery in biomedical fields. Moreover, the biological agents based on the biosources offer various advantages for treatment of different diseases in a safe manner.[45-54]

Conclusions

The drug ciprofloxacin in PLGA NPs was easily encapsulated by using a novel, drop wise, one step method. Nanoparticles of desired size were successfully formed using polymer such as PLGA. NPs with considerable drug-loading efficiency were formed with PLGA 50; 50. PLGA loaded NPs showed a sustained release of drug ciprofloxacin. The obtained results revealed that usage of ciprofloxacin in this way increases the gastric time retention and residence as well as enhances the bioavailability. Simultaneously, it also decreases the dosage quantity and dosage time interval. So conclusively, it can be said that nanoparticles of ciprofloxacin can be used for lengthy and long times of release of drug in stomach which can at least be 8 h without taking next dose. By optimization of drug and polymer ratios, different and novel treatments can be achieved in future applications.

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ORCID iDs

Samar Z. Alshawwa https://orcid.org/0000-0001-9232-7956
Munawar Iqbal https://orcid.org/0000-0001-7393-8065

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