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PLGA Nanoparticles for the Oral Delivery of 5-Fluorouracil Using High Pressure Homogenization-Emulsification as the Preparation Method and In Vitro/In Vivo Studies

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The objective of the present study was to incorporate the hydrophilic anti-cancer drug 5-Fluorouracil(5-FU) into poly(lactide-co-glycolide) (PLGA) nanoparticles(NP) to improve the oral bioavailability. Owing to the high solubility of 5-FU in basic water, the water-in-oil-in-water (w/o/w) emulsification process has been chosen as one of the most appropriate method for the encapsulation of 5-FU, and the ammonia solution was used as the inner aqueous phase solvent to increase the solubility of 5-FU. In order to reach submicron size as well as increasing the grade of monodispersity compared to previous preparation techniques, we prepared 5-FU loaded PLGA-NP by a high-pressure emulsification-solvent evaporation process. The PLGA-NPs were characterized with respect to their morphology, particle size, size distribution, 5-FU encapsulation efficiency, in vitro and in vivo studies in rats. In vitro release of 5-FU from nanoparticles appeared to have two components with an initial rapid release due to the surface associated drug and followed by a slower exponential release of 5-FU, which was dissolved in the core. The in vivo research was studied in male Sprague-Dawley rats after an oral 5-FU dose of 45 mg/kg. Single oral administration of 5-FU loaded PLGA-NP to rats produced bioavailability, which was statistically higher than 5-FU solution as negative control. And the MRT (mean residence time) of 5-FU loaded PLGA-NP was significantly ($P < 0.05$) modified. Thus, it is possible to design a controlled drug delivery system for oral 5-FU delivery, improving therapy efficiency by possible reduction of time intervals between peroral administrations and reduction of local gastrointestinal side effects.

Keywords 5-Fluorouracil; PLGA; W/O/W emulsion; nanoparticles; high pressure homogenization; drug release

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INTRODUCTION

In the field of controlled drug delivery system, increasing attention is focused on biodegradable polymers such as poly(DL-lactic-co-glycolic acid)(PLGA) because of its biodegradability and biocompatibility (Lampretch, Ubrich, Yamamoto et al., 2001). PLGA are widely employed for the preparation of sustained release preparations (Schachter & Kohn). Especially nanoparticles(NP) delivery systems made of PLGA provide an attractive alternative for long term delivery of therapeutic agents for chronic administration (Chong, Cao, Wong et al., 2005). PLGA-NP are colloidal polymeric drug carriers that hold promise for peroral drug delivery which represents by far the most common and convenient route of administration. These PLGA-NP offer many advantages over conventional oral dosage forms, such as enhancing the oral bioavailability of those poorly-absorbed drugs, protecting the encapsulated drugs in the polymer network.

Several studies done at the tissue and cellular levels have demonstrated that PLA and PLGA copolymer particles in a size range of 50 nm to 20 μm are absorbed mainly through the Peyer's patches found in small intestine (McClean, Prosser, Meehan et al., 1998; Shakweh, Besnard, Nicolas et al., 2005). These studies have also explained that translocation is largely dependent on particle size: smaller particles are more readily absorbed. Additionally, the oral bioavailability of the drug was improved by its incorporation into polymeric NP (Gutierro, Hernandez, Igartgua et al., 2002).

Several encapsulation techniques have already been developed to prepare particulate sustained drug delivery systems. Some of the commonly reported methods of preparing nanoparticles from biodegradable polymers include emulsion solvent

evaporation (Budhian, Siegel, & Winey, 2005), monomer polymerization (Peng, Cheng, & Zhuo, 2006), nanoprecipitation (Govender, Stolnik, Garnett, et al., 1999), cross-flow filtration (Quintanar-Guerrero, Ganem-Quintanar, Allemann et al., 1998) or emulsion-diffusion technique (Konan, Cerny, Favet et. al., 2003) and the salting out procedure (Avgoustakis, 2004). However, the choice of a particular method of encapsulation is usually determined by the solubility characteristics of the drug. Many authors (Bilati, Allemann, & Doelker, 2005; Blanco & Alonso, 1997; Singh, Pandit, Bramwell, & Alpar, 2006) have previously shown that the double emulsion technique was the most appropriate method to encapsulate hydrophilic drugs within NP. In these cases the homogenization of the double emulsion was performed by sonication. However, in each cases the polydispersity of the NP size remained relatively high.

The present study provides an improved method for producing 5-Fluorouracil (5-FU) loaded PLGA-NP. We prepared the double emulsion with a lower polydispersity of the size by using a high pressure homogenization device for the 5-FU loaded PLGA-NP preparation. 5-FU is more dissoluble in basic water than in distilled water, thus ammonia solution was selected as the inner aqueous phase solvent to increase the solubility of 5-FU so as to increase the encapsulation and drug loading of the PLGA-NP. The physicochemical characteristics, particle morphology, in vitro and in vivo release behavior of 5-FU loaded PLGA-NP have been elucidated. The effect of various formulation parameters on the properties of the 5-FU loaded PLGA-NP have been studied, and the underlying mechanisms behind various aspects related to the physical characteristics of 5-FU loaded PLGA-NP have also been evaluated and interpreted.

MATERIALS AND METHODS

Materials

Poly(D,L-lactic-co-glycolic acid) (PLGA, molar ratio of D,L-lactic to glycolic acid, 75:25, MW = 15 kDa) were purchased from Shandong Key Laboratory of Medical Polymer Materials (Shandong, China). Poloxamer 188 (F-68, MW = 8350 ± 1000) of medical grade was a gift from the Surfactant Institute of Jinling Petrochemical Co. (Nanjing, China) and chosen as a surface active agent for the second emulsion. 5-Fluorouracil (5-FU)was purchased from Jiangsu Nantong Jinghua pharmaceutical Plant (Jiangsu, China)and used as the model drug. Double-distilled water was filtered through a 0.45 μm (cellulose acetate) membrane before use. All other chemical reagents and solvents used were analytical or spectroscopic grade.

Preparation of 5-FU-PLGA-NP

Nanoparticles (NP) were prepared by using the multiple emulsion (w/o/w) technique, previously applied to the preparation of both micro- and nanoparticles (Blanco & Alonso, 1997; Singh, Pandit, Bramwell, & Alpar, 2006). It involves two major steps, the formation of stable droplets of the first emulsion and the subsequent removal of solvent from the droplets of the second emulsion. However, the polydispersity index still remains relatively high. In our study, the choice of the nanoparticle preparation consisted in a high pressure homogenization process which allowed to reduce considerably the mean particle size and simultaneously to narrow the width of the size distribution, i.e. reduce the polydispersity index (Arbos, Campenero, & Irache, 2002). The methodology in brief goes as follows: the 5-FU previously dissolved in 0.6 mL of distilled water (adding ammonia water to a pH value of 11.0) was emulsified with 3 mL of acetic ether containing PLGA (6% w/v) for 1 min using an ultrasonic probe (JY88-II, Zhejiang, China). This first emulsion (w/o) was then added to 12 mL poloxamer 188 solution (1.0%) using a stirring motor with a stainless-steel propeller (F6/10, FLUKO, German) and the resulting coarse pre-emulsion was immediately passed through a high-pressure homogenizer (Panda 2K, SEEKER, Canada) three times at an operating pressure of 800 bar in an ice bath for three times. The double emulsion was diluted in 120 mL poloxamer 188 solution (2.0%, w/v) and the acetic ether was rapidly eliminated under reduced pressure at 40°C, the NP were formed when the PLGA precipitated and the NP were collected by centrifugation at 70,000 × g for 30 min (5810R, Eppendor, Germany). The obtained PLGA-NP were washed with Milli-Q water and recentrifuged three times before lyophilization. At last, two percent (2% w/v of nanosuspension) mannitol was used as cryoprotectant. Five milliliters of the filtered nanosuspension was quickly snap frozen in liquid nitrogen at –45°C for 6 hr and lyophilized for 36 hr (LGJ-10, Beijing, China) at –20°C and pressure of 60 Pa. The freeze-dried powder was collected for later use.

The encapsulation efficiency was determined by measuring the non-encapsulated amount of 5-FU in the supernatant recovered after ultracentrifugation and washing of the NP. The effect of several variables on the characteristics of the NP was evaluated, including the concentration of 5-FU (40, 45, 50, 55, and 60 mg/mL), poloxamer 188 (0.5, 1.0, 1.5, 2.0, and 2.5%), PLGA(30, 40, 50, 60, and 70 mg/mL), and the volume ratio of external aqueous phase to organic phase (22, 44, and 66).

5-FU Loaded PLGA-NP Morphology

The morphological of the 5-FU loaded PLGA-NP was ascertained by Transmission Electron Microscopy (TEM) (JEM-200CX, JEOL, Japan). A drop of the 5-FU loaded PLGA-NP suspension (10 mL) was placed on copper electron microscopy grids (Formvar filmed) and stained with a 2% (w/v) phosphotungstic with fresh acid solution. After 30 sec the sample was washed with ultra-purified water and the excess fluid removed with a piece of filter paper. The dried sample was then examined.

Particle Size Analysis

The average particle size, polydispersity, and size distribution profiles were determined at 25°C by photon correlation spectroscopy (PCS) with a Zetasizer (Malvern 3000 HAS Malvern Instruments, Malvern) after being diluted 10 times with distilled water. Multimodal type of analysis was adopted for particle size distribution. Each value is a mean of three measurements of 120 sec each, divided into 10 sub runs. The diameter is calculated from the auto correlation function of the intensity of light scattered from the particles assuming a spherical form of particles. Each sample was measured in triplicate and an average particle size was expressed as the mean diameter ± standard deviation.

Encapsulation Efficiency and Drug Loading

The encapsulation efficiency of the PLGA-NP was defined as the percentage of 5-FU encapsulated in respect to the total amount of 5-FU used to prepare the PLGA-NP and the drug loading was defined as the percentage of 5-FU encapsulated in respect to the total amount of the 5-FU loaded PLGA-NP. The amount of 5-FU entrapped within the PLGA-NP was determined by measuring the amount of non-entrapped 5-FU by ultraviolet spectroscopy at 265 nm that was recovered in the supernatant after ultracentrifugation(5810R, Eppendor, German) and wash of the 5-FU-PLGA-NP for three times at 70,000 × g for 30 min Each sample was assayed in triplicate and the results were reported as average ± standard deviation.

In Vitro 5-FU Loaded PLGA-NP Release Studies

In vitro release profiles of 5-FU from the PLGA-NP were obtained by a dissolution test in phosphate buffer solution (PBS, release medium, pH 7.4). In order to keep the system under sink conditions during the release studies. the amount of drug-loaded PLGA-NP was chosen such that the 5-FU content of the PLGA-NP was lower than 10% of the saturation concentration of 5-FU(34.72 mg/mL). The in vitro release experiment was carried out as follows: 60 mg lyophilized 5-FU loaded PLGA-NP and 2 mL PBS (pH 7.4) was placed into dialysis bag that immersed into 50 mL PBS solution and the system was placed in an orbital shaker bath, which was maintained at 37 ± 0.1°C and shaken horizontally at 100 min^{-1} . At predetermined intervals, aliquots of the release medium (5 mL) was taken out and assayed for drug release and replaced by 5 mL of fresh buffer at each sampling point and agitation was continued. 5-FU in the release medium was quantitated by UV spectrophotometry at 265 nm since PLGA showed no significant absorption at this wavelength and accumulative release weight of 5-FU was calculated according to the calibration curve. All release experiments were performed in triplicate. The cumulative amount of 5-FU release from the 5-FU loaded PLGA-NP was calculated using the following equation:

$$\text{Cumulative \% release} = Mt/Mactual \times 100$$

where, Mt is the amount of 5-FU released from the PLGA-NP at time (*t*) and Mactual is the actual amount of 5-FU present in the PLGA-NP. The cumulative percentage of 5-FU released is plotted versus time. Each data point is mean ± *S. E.* calculated on three measurements.

Blood-Level Studies

Twelve male albino rats of Sprague–Dawley strain of similar weights (250 ± 20 g) were used throughout the in vivo studies. The rats were requested from the Experimental Animal Center of Southeast University randomly divided into two groups (six animals each). They were acclimatized in well-spaced ventilated cages at a temperature of 25 ± 2°C and a relative humidity of 70 ± 5% under natural light /dark conditions for 4 days and free access to standard food and tap water. The animals were fasted overnight before experiments but allowed water ad libitum. The 5-FU solution and 5-FU loaded PLGA-NP were given orally to rats in a single administration by using a feeding needle at a 5-FU dose of 45 mg/kg. Group 1 received 5-FU solution and group 2 received 5-FU loaded PLGA-NP. 0.1 mL of plasma samples were withdrawn from the retro-orbital plexus of the rats using uniformly tapered capillary at the certain times as shown in Table 3. Samples were collected in polyethylene vials over 20 μL heparin sodium (22 mg/mL) and separated by centrifugation at 3000 × g for 10 min at 4°C and the plasma was frozen at –20°C until analyzed. Aliquots (0.2 mL) of the plasma were extracted with EtAc. The protein precipitate was removed by centrifugation at 5000 × g for 10 min at 4°C. The supernatant was decanted, evaporated to dryness using N_2 flow and re-constituted in assay buffer (0.1 mL). Standard curves to quality 5-FU uptake in vivo were prepared by vortexing PABA standards with blood samples, followed by plasma preparation and extraction as describe above.

The amount of 5-FU in rats was determined by reversed-phase high-performance liquid chromatography (RP-HPLC) at a detection wavelength of 265 nm, The HPLC system consisted of a quadr-piston pump (P680A, Dionex), and a UVD-170U variable wavelength UV detector (UVD170UV-VISDionex). Separation was achieved by using a reversed-phase column(Kromasil C18, 150 × 4.6 mm ID, 5 mm particle size, Dikma) protected by a guard column (4.6 × 50 mm) of the same material and thermostated at 30°C and a flow rate of the mobile phase of 0.8 mL/min. The mobile phase consisted of methanol/20 mM KH_2PO_4 (5:95, v/v, pH 5.5). Calibration curves in blood sample matrix were obtained by analyzing a set of samples. Under these conditions, percentage recovery of 5-FU in the blood was 100 ± 5% and the within-day and between-day coefficients of variation did not exceed 5% for the same batch of reagents. The limit of quantification was in all cases 25 ng/mL and no interference of the compounds used in NP preparation was observed.

The plasma drug concentration over time data was used to calculate pharmacokinetic parameters. The pharmacokinetic

parameters were estimated by two-compartmental methods. The peak plasma concentration (C_{max}) was obtained by visual data inspection. The area under plasma drug concentration over time curve (AUC) was obtained by the trapezoidal rule. The differences found between pharmacokinetic parameters in both groups were statistically evaluated by the *t*-test. The results from the in vivo rats study were expressed as the mean ± standard error for 12 rats.

RESULTS AND DISCUSSION

Several studies have demonstrated an improved bioavailability of drugs encapsulated in nanoparticles by oral administration (Bala, Bhardwaj, Hariharan et al., 2006; Hariharan, Bhardwaj, Bala, Sitterberg, et al., 2006). PLGA-NP coated either with albumin, an easily digestible protein, or poloxamer 188, a non-digestible synthetic polymer, have already been evaluated as a potential carrier system for oral administration (Hariharan et al., 2006). These nanoparticles can be easily prepared by high-pressure homogenization emulsification-solvent evaporation (HPESE) process.

The prepared PLGA-NP should be hydrophilic due to the poloxamer 188 coating around it. Poloxamer 188 is a swellable, hydrophilic polymer and therefore the higher amount of residual poloxamer 188 at the surface of the PLGA-NP would account for their hydrophilicity (Dong & Feng, 2005). And the large amount of poloxamer 188 coat around the PLGA-NP would form a hydrogel barrier to control the diffusion release of 5-FU from the PLGA-NP.

Morphology of 5-FU loaded PLGA-NP was observed by TEM (Figure 1) and the image demonstrated that NP appear spherical with a relatively monodispersed size.

In of the instrument used (Malvern 3000 HAS Malvern Instruments, Malvern), the size distribution was specified in the intensity of the dynamic light scattering and the polydispersity was defined as the log normal distribution width of the particle diameter. The size distribution graph is presented in Figure 2. The graph indicates that over 90% of the particles are in the range of 75.4–102.6 nm with a mean value of 85.4 ± 4.2 nm. The size distribution was narrow with polydispersity of 0.10 to 0.18. Thus, a high-pressure homogenizer appears to be an effective instrument to produce PLGA-NP with a narrow size distribution.

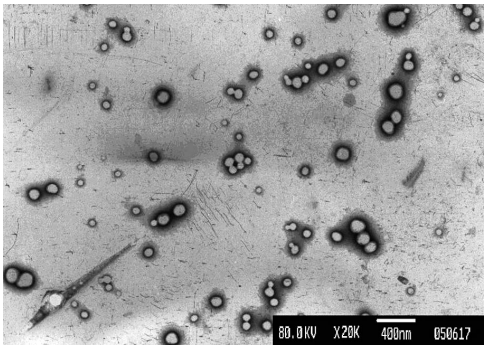

FIGURE 1. Transmission electron microphotograph 5-FU loaded PLGA-NP prepared by multiple emulsion (W/O/W) technique combined with high pressure homogenization ($\times 2 \times 10^4$).

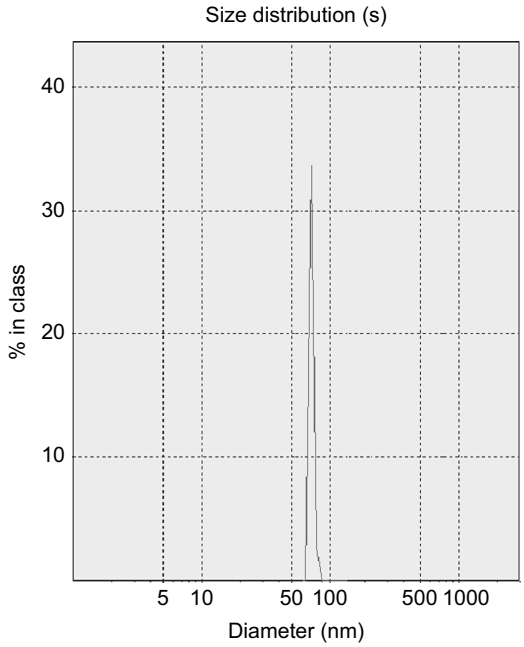

FIGURE 2. The size distribution of 5-FU loaded PLGA-NP by dynamic light scattering (based on the number of the PLGA-NP).

Effect of 5-FU Concentration

As reported in Table 1, NP size and polydispersity were only slightly affected by the amount of the drug since no significant differences were observed for PLGA-NP of different amount of the drug. On the other hand, after the second emulsion, the organic solvent starts to evaporate, and the PLGA precipitates at the interface between the external aqueous phase and the organic one. When the polymer does not precipitate

TABLE 1
Influence of the 5-FU Concentration in the Inner Aqueous Phase on the Particle Size and the Polydispersity

5-FU Concentration (mg/mL)	Size (nm)	Polydispersity
40.0	83.2 ± 3.9	0.112 ± 0.052
45.0	81.4 ± 7.0	0.098 ± 0.035
50.0	85.4 ± 4.2	0.186 ± 0.044
55.0	85.3 ± 6.4	0.138 ± 0.057
60.0	87.7 ± 5.2	0.115 ± 0.064

Data are shown as $M \pm S.E.$ obtained from three formulations.

rapidly at the outer surface, diffusion of 5-FU into the aqueous phase before the formation of NP can take place. In spite of its low solubility in the organic polymer solution, this phenomenon could involve a part of the loaded 5-FU to relocate at the NP surface.

Figure 3 shows that the concentration of 5-FU in the internal aqueous phase had a non-negligible influence on the encapsulation efficiency (EN) and drug loading (DL). The results show that the optimal encapsulation efficiency reached about 64% for PLGA-NP when 50 mg of 5-FU were used, and by increasing 5-FU concentration encapsulation efficiencies diminished down to a plateau but the drug loading increased. It should also be noted that even at low loading 5-FU concentration, a fraction of the drug was not encapsulated during the preparation of NP. There are several possible explanations of the loss of 5-FU, which was not entrapped within NP. Among them, the difference in osmotic pressure between the internal and external aqueous phases could be responsible for the decrease in encapsulation efficiency. The osmotic pressure difference did in fact increase with increased 5-FU loading, leading to a rupture of the lipophilic droplets, and an exchange between the internal and external aqueous phases, with a consequent loss of 5-FU. Moreover, it is also possible that the polymer does not precipitate quickly enough and a loss of 5-FU occurs. Indeed, Bodmeier and McGinity (1988) reported that the successful encapsulation of drugs within particles is dependent on the fast precipitation of the coating polymer from the organic phase. The removal of the organic solvent under reduced pressure favors its fast evaporation followed by the polymer precipitation, thus reducing the migration of the drug to the external phase. In addition, theoretically, during the formation of the particles by the double emulsion: solvent evaporation method, the polymer solution acts as a barrier between the internal and external aqueous phases. Nevertheless, an exchange between the two aqueous phases might occur as a consequence of the instability of the primary emulsion, and particularly because of 5-FU solubility in the outer aqueous phase.

Effect of PLGA Concentration

By increasing the PLGA concentration in the organic phase an increase of the size as well as the polydispersity of 5-FU loaded PLGA-NP was observed (Figure 4). In addition, when the PLGA:5-FU ratio was increased by increasing the amount of PLGA in the organic phase, an increase in 5-FU encapsulation within NP and the drug loading of the PLGA-NP was observed (Figure 5). These results could be explained that an increase in PLGA concentration led to an increase of the viscosity of the

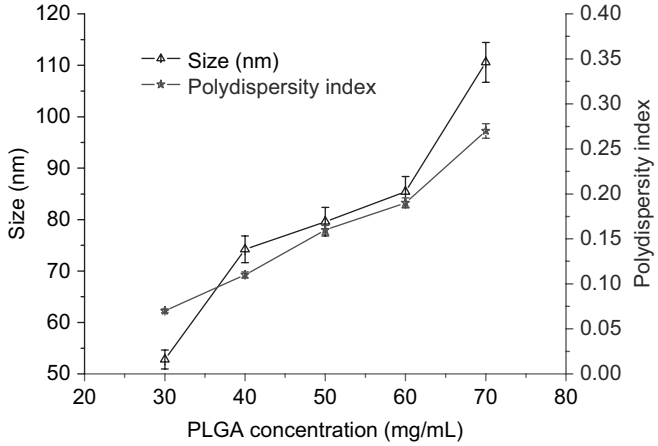

FIGURE 4. Effect of the PLGA concentration in the organic phase on the size (Δ) and polydispersity (★) of PLGA-NP. Data are shown as $M \pm SE$ obtained from three formulations.

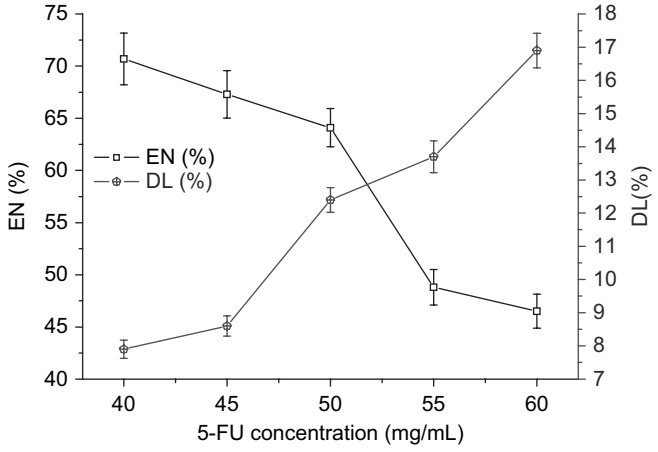

FIGURE 3. Encapsulation efficiency and drug loading of 5-FU loaded PLGA-NP versus the concentration of 5-FU dissolved into the internal aqueous phase. Data are shown as $M \pm SE$ obtained from three formulations.

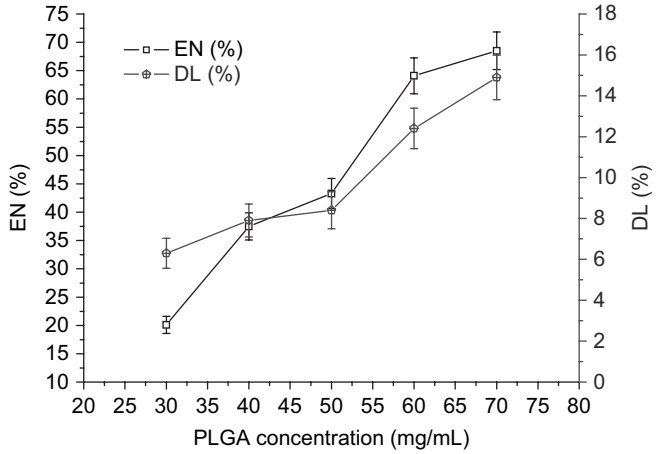

FIGURE 5. Encapsulation efficiency and drug loading of 5-FU loaded PLGA-NP versus the concentration of PLGA in the organic phase Data are shown as $M \pm SE$ obtained from three formulations.

first emulsion which might prevent the optimal shearing of the emulsion when agitated and consequently to a reduction in the partitioning of the drug into the external aqueous phase, resulting in an increase in 5-FU encapsulation efficiency.

At the same time, the first emulsion subsequently led to less efficient reduction of the emulsion droplet size during the second emulsification step. Provided that emulsification at the same condition produces nanodroplets of similar dimensions, decreasing PLGA concentration leads to the formulation of smaller nanoparticles, since PLGA content of the individual droplets decreases. Additionally, lowering the PLGA concentration diminishes the viscosity and leads to formation of smaller droplets. As showed in Table 2, When the volume of the organic phase was increased stepwise from 6 to15 mL in the presence of a constant amount (600 mg) of PLGA, the mean diameter of drug-loaded nanoparticles decreased from 162.8 to 75.4 nm. In contrast, no evidence of size altering was observed upon variation of the volume of the organic phase, when the concentration of the PLGA was held constant.

Effect of the Volume of the External Aqueous Phase

An increase in the volume of the external aqueous phase led to an increase in both the encapsulation efficiency and size of the 5-FU loaded PLGA-NP (Figure 6). The increase in particle size was probably attributed to a reduction of the shear during the homogenization process, because of a decrease in mixing efficiency associated with larger volume, involving an increase of the size of the emulsion droplets and consequently of the NP.

Effect of Poloxamer 188 in the External Aqueous Phase

Since exchanges between the internal and external aqueous phases should be kept to a minimum during the second emulsification step, the stability of the second emulsion is also critical. Furthermore, during the solvent evaporation process, there is a gradual decrease in the volume and subsequent increase in the viscosity of the dispersed droplets. This affects the droplets size equilibrium, involving the coalescence and the agglomeration of the droplets during the early step of the solvent removal.

TABLE 2
Influence of the PLGA Concentration and the Volume of the Organic Phase on the Particle Size

Volume (mL)	PLGA Concentration (mg/mL)	Mean Diameter (nm)
6.0	100.0	162.8 ± 15.2
10.0	60.0	88.7 ± 4.2
15.0	40.0	75.4 ± 7.8
15.0	60.0	88.1 ± 7.2
20.0	60.0	84.6 ± 5.4

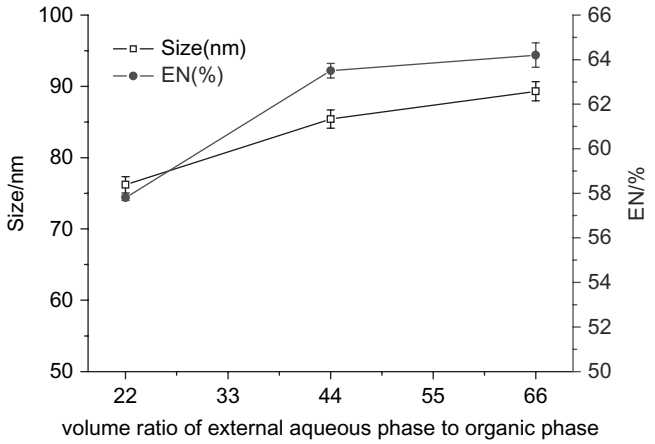

FIGURE 6. Effect of the volume ratio of external aqueous phase to organic phase on the mean diameter and encapsulation efficiency(EN) of 5-FU loaded PLGA-NP. Data are shown as *M* ± *SE*.

This problem has been improved by adding a surfactant into the continuous phase, providing a thin protective layer around the droplets and hence reducing their coalescence. While maintaining a constant volume for the external aqueous phase (100 mL), the amount of poloxamer 188 was varied. As reported in Figure 7, the size and the polydispersity of PLGA-NP decrease with increased poloxamer 188 concentrations. It could thus be concluded that the more the poloxamer 188 concentration increased, the more the poloxamer 188 molecules covered the interface between organic phase and external aqueous phase. An improved protection of the droplets from coalescence was obtained, leading consequently to smaller emulsion droplets than at lower poloxamer 188 concentrations. Since NP was formed from the emulsion droplets after the solvent evaporation, their size is dependent upon the size and the stability of the emulsion droplets.

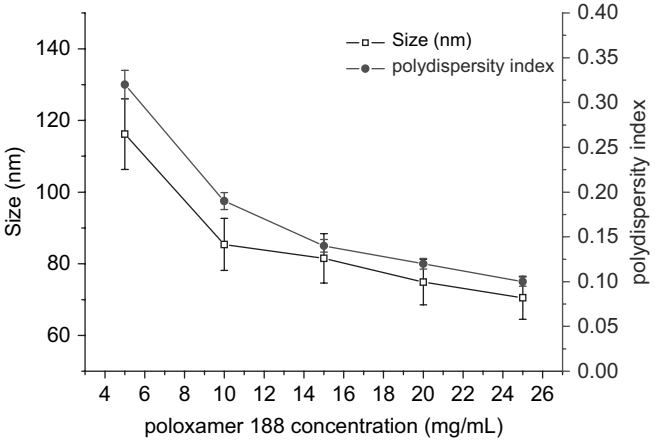

FIGURE 7. Effect of the poloxamer 188 concentration in the external aqueous phase on the size and the polydispersity of PLGA-NP. Data are shown as *M* ± *SE*.

In Vitro Release of 5-FU Loaded PLGA-NP

Figure 8 illustrates the in vitro release profile of 5-FU loaded PLGA-NP, in pH 7.4 phosphate buffer, by representing the percentage of 5-FU release with respect to the amount of 5-FU encapsulated. The saturation concentration of 5-FU in pH 7.4 phosphate buffer is 34.72 mg/mL which definitely demonstrates that the sink conditions (defined as 30% of the saturation concentration) were maintained during the whole dissolution experiment. Therefore the non-complete release cannot be explained by non-sink conditions.

Figure 8 illustrates the in vitro release profile obtained for 5-FU loaded PLGA-NP. The profile was characterized by an initial and variable rapid release period followed by a continuous and slower release thereafter, and the in vitro release profile showed a three-phase composition:

1. A first rapid initial burst release, owing to the drug located on or near the surface of the delivery system and the large surface to volume ratio of the nanoparticles geometry because of their size and therefore available for immediate release.
2. After the initial burst, the drug release profiles displayed a plateau for an certain period, resulting from the only diffusion of the drug dispersed into the polymer matrix;
3. A constant sustained release of the drug over 10 days resulting from the diffusion of the drug through the polymer wall as well as its erosion.

When particles are prepared by the w/o/w method, water-soluble drugs exhibit a tendency to migrate to the aqueous medium, thereby concentrating at the surface of the particles and involving the burst effect. Moreover, the burst release could also be explained by the imperfect encapsulation of the drug inside nanoparticles, resulting from the unstable nature of the emulsion droplets during the solvent removal step. This potential instability may cause a part of the loaded drug to relocate at the nanoparticle surface, thereby rapidly released.

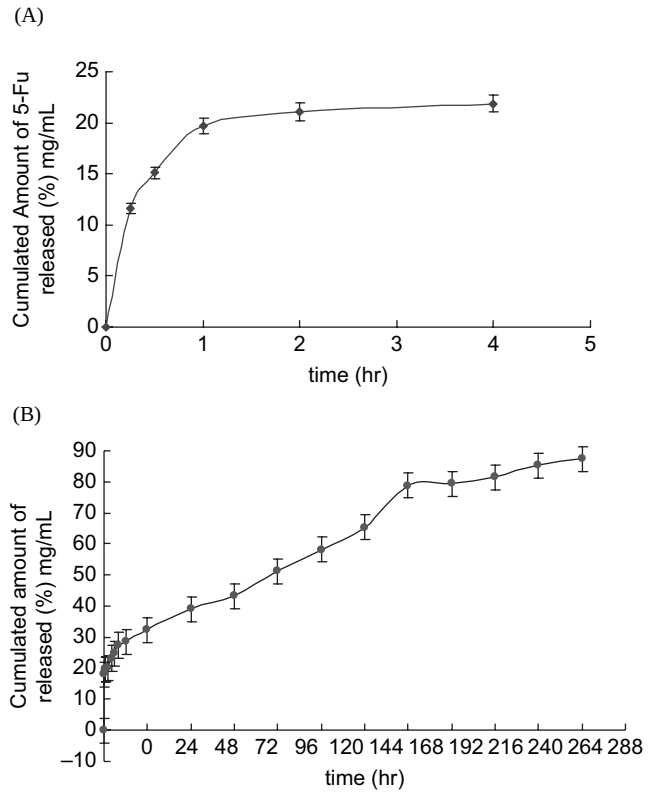

FIGURE 8. Release profiles of 5-FU from PLGA-NP in phosphate buffer at 37 °C and pH 7.4 after 4 hr (A) and 11 days (B). Data are shown as $M \pm SE$.

In Vivo Evaluation of 5-FU Loaded PLGA-NP

Blood-level studies were taken up to determine the release and performance of the 5-FU loaded PLGA-NP in vivo. The PLGA-NP was precentrifuged to remove unentrapped drug and the amount equivalent to 11.25 mg 5-FU was administered to the SD rats by i.g. route. The plasma was used to determine the concentration of 5-FU in blood samples at various time intervals. Similar to the release rate trend found in vitro, the blood level of the drug by i.g. route was found to be lower and somewhat constant in case of 5-FU-PLGA-NP than that of free drug solution due to slower release rate of the drug as shown in Table 3. The blood data also reveal that the 5-FU-PLGA-NP reduces the rapid clearance from the circulation.

Table 4 shows the corresponding pharmacokinetic parameters derived by two-compartmental analysis. The C_{max} from free drug solution of 5-FU was found nearly 19.75 μg/mL and t_{max} occurred at 0.26 hr after i.g. route and was detected up to 3–4

TABLE 3
Blood-level Study of 5-Fluorouracilsolution and 5-FU Loaded PLGA-NP in SD Rats After i.g. Route

	Blood Concentration (μg/mL)	
Time (hr)	5-FU	5-FU-PLGA-NP
0.083	7.32 ± 2.74	2.52 ± 0.68
0.25	21.89 ± 4.47	8.95 ± 2.94
0.5	5.40 ± 1.71	4.83 ± 2.42
1.0	7.86 ± 2.64	11.92 ± 2.92
1.5	4.36 ± 1.43	/
2.0	3.57 ± 2.18	5.85 ± 2.19
3.0	1.35 ± 0.80	/
4.0	1.02 ± 0.34	2.59 ± 1.75
6.0	Undetectable	/
8.0	—	2.16 ± 1.01
12.0	—	1.49 ± 0.67
24.0	—	1.09 ± 0.55
36.0	—	Undetectable

TABLE 4
Various Pharmacokinetic Parameters of 5-Fluorouracil as Determined on Administration of Different Formulations ($P < 0.05$)

Parameters	5-FU	5-FU-PLGA-NP
C_{max} (μg/mL)	19.75 ± 4.31	10.93 ± 2.52
T_{max} (hr)	0.26 ± 0.11	1.13 ± 0.21
$t_{1/2}$	0.36 ± 0.09	2.35 ± 0.42
AUC (h•μg/mL)	18.15 ± 4.14	41.09 ± 9.05
MRT	1.52 ± 0.37	6.63 ± 1.03

hr, whereas, C_{max} from 5-FU-PLGA-NP was found nearly 10.93 μg/mL and t_{max} occurred at 1.13 hr after i.g. route. The blood level was much prolonged and was detectable up to 24 hr. The formulations were found to be following sustained release characteristics for 5-FU as shown by the relative increase in MRT (mean residence time) for 5-FU-PLGA-NP as compared to plain drug solution (4.36 times). The pharmacokinetic analysis revealed a significant increase ($P < 0.05$) in MRT, $t_{1/2}$, AUC as well as relative bioavailability of encapsulated drugs compared with free drugs after treatment with the same dose.

The mean pharmacokinetic profiles obtained for each group receiving 5-FU orally as PLGA-NP of 85.4 nm are depicted in Figure 9. The release rate however, was found to have increased in vivo as compared to in vitro data, possibly due to the metabolism by the enzymes and hydrolysis in body.

The nanoparticle formulations have been known to be an efficient approach to achieve better pharmacokinetic profiles due to their specialized uptake mechanisms: the nanoparticles remain in the blood circulation for a longer time and release drug in the plasma in a sustained and continuous manner leading to lower fluctuations in the blood levels that could minimize adverse effects caused by the drug. It is also shown that PLGA has bioadhesive properties and binds with the mucosa of the gastrointestinal tract. This may increase the residency time of the sustained release formulation and may enhance the drug absorption due to intimacy of contact with the epithelium cells. In addition, the nanoparticles can naturally be taken up by enterocytes and/or the lymphoid tissues in Peyer's patches (PPs) (Shakweh, Besnard, Nicolas et al., 2005), so the bioavailability of the 5-FU PLGA nanoparticle formulation is increased obviously.

CONCLUSION

In this paper, 5-FU-loaded PLGA-NP were prepared successfully by water-in-oil-in-water emulsion followed by HPESE process which produces small, monodisperse PLGA-NP combined with easy control, and reproducibility. The process can be automated and scaled up for producing large amount of PLGA-NP required for animal and human studies. Consequently, this new approach of using biodegradable nanoparticles as 5-FU carriers is a promising step toward the development of a clinically useful new dosage form of this drug.

The encapsulation efficiency of the 5-FU loaded PLGA-NP is only about 64%. This may be attributed to the water soluble nature of 5-FU which led to its rapid partitioning into the aqueous phase and hence decreased encapsulation into the nanoparticles during PLGA deposition. The large surface to volume ratio the nanoparticle geometry may have also contributed to loss of drug into the aqueous phase during preparation.

By in vitro and blood-level studies. The present NP can be expected to be suitable for prolonged delivery of hydrophilic compounds such as 5-FU prepared by high pressure homogenization. Moreover, the preliminary 5-FU release test from the PLGA-NP in vitro proved that the present NP had the properties of a sustained release form.

Consequently, with the use of PLGA as an excipient for drug formulation it is possible t design a biodegradable controlled-

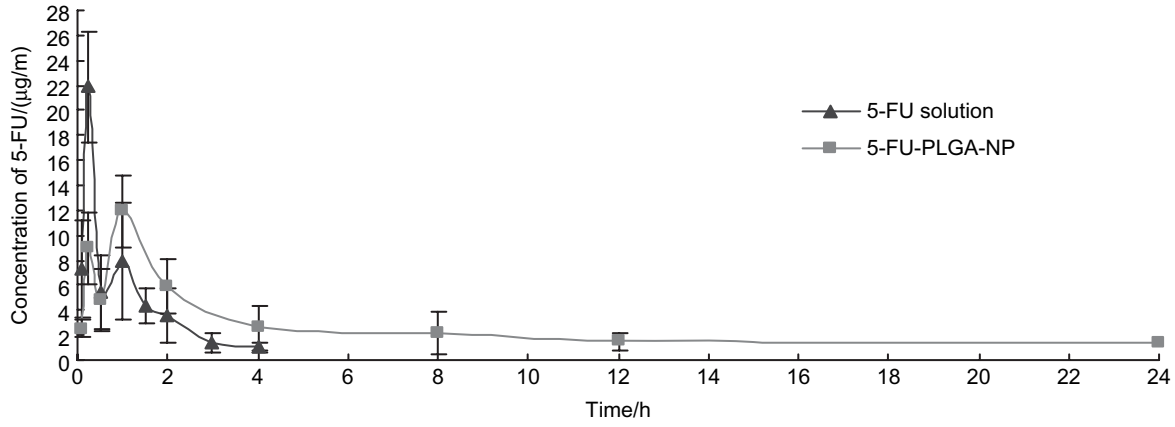

FIGURE 9. Whole blood concentration ± time profiles of 5-FU ($M \pm SE$, $n = 6$) following a single 45 mg/kg oral administration to male *SD* rats as drug-loaded PLGA-NP.

release system which, after a single dose, can maintain the drug at the desired concentrations, achieving the ideal mode of 5-FU delivery which showed as an initial burst ('induction therapy' phase), followed by a prolonged continuous release, to attain a 'maintenance therapy' phase. Additionally, the oral bioavailability of this drug was improved by its incorporation into PLGA-NP due to the small surface area of the PLGA-NP.

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