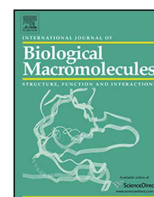
International Journal of Biological Macromolecules 95 (2017) 643–649

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

journal homepage: www.elsevier.com/locate/ijbiomac



# Preparation and characterization of polymeric nanoparticles surface modified with chitosan for target treatment of colorectal cancer

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ARTICLE INFO

*Article history:*
Received 31 October 2016
Received in revised form
14 November 2016
Accepted 23 November 2016
Available online 28 November 2016

*Keywords:*
Cytotoxicity
5-Fluorouracil
Nanoparticles

ABSTRACT

5-Fluorouracil (5-FU) loaded chitosan (C) coated polylactic-co-glycolic acid (PLGA) nanoparticles [NPs] (C-5-FU PLGA NPs) and polycaprolactone [PCL] (C-5-FU PCL NPs) were employed as the carriers for cancer treatment. The prepared NPs showed the spherical shape of NPs with the particle size in the range of 188.1–302.2 nm with polydispersity index (PDI) of <0.30. C-coated NPs converted zeta potential from negative to positive value with small modification in particle size distribution. The entrapment efficiency of 5-FU was recorded in the range of 32–51%. The *in vitro* release studies showed an initial rapid 5-FU release followed by a sustained release profile. The *in vitro* cytotoxicity of C-5-FU PLGA NPs showed significant inhibition of colon cancer cells (HT-29) compared to the other NPs and drug solution. These results showed that C-5-FU PLGA NPs can be considered as a promising carrier for cancer therapy.
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## 1. Introduction

The nanoparticles (NPs) for targeting of chemotherapeutic agents are considered a very hot field for cancer treatment [1,2]. Polymeric NPs were considered as an interesting area for delivery of small molecules to overcome the poor drug solubility and cell permeability [3]. More concern has been dedicated on the natural and synthetic polymers such as poly-caprolactone (PCL) and poly (lactide-*co*-glycolide) (PLGA) due to their good biodegradablity, biocompatibility and extend drug release profile [4]. These polymers have been shown to shield the drug molecules from its degradation by enzymes and offer physicochemical stability [4,5].

Nanoparticles (NPs) can be formulated with optimum size and shape to enhance their drug release and cellular uptake [5]. PLGA NPs have been extensively employed to target many anticancer drugs through attachment of specific ligands to the surface of the particles [6]. PCL NPs have been extensively used in many drug delivery systems due to their good solubility, wide mixture compatibility and superior permeability [7]. In addition, PCL possesses eminent rheological properties and can be flexibly configured to many shapes allowing for a broad range of biomedical applications [8]. Moreover, the surface of NPs can be modified with ligands to improve cellular drug delivery [9].

Chitosan (C) has received increasing attention as a good polymeric material and has now been broadly used in several fields such as protein adsorption [10] and metal adsorption [11]. Additionally, C has also been studied in the development of extended release drug delivery systems [12]. Since C has mucoadhesive character, which enhances drug absorption and promote prolongation of drug release.

Surface modification of NPs with C has numerous pharmaceutical benefits. The coating by C decreases the burst result of drug release. The positively-charged C efficiently attracts to the negatively charged of the membrane, which enhances the retention and permeation of NPs [13].

5-Fluorouracil (5-FU) has a broad range of anticancer activity, including those of the colorectal region, liver, pancreas, lung and breast [14]. It has been reported as hydrophilic drug (saturated solubility in water has been reported as 10–12 mg/ml) [15,16]. It had very low oral bioavailability (sometimes unpredictable after oral administration) due to erratic *in vivo* absorption [17–19]. The traditional systemic administration by the intravenous route needs relatively high doses of FU, which results in the high profile of hematologic and bone marrow toxicities [20].

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http://dx.doi.org/10.1016/j.ijbiomac.2016.11.098
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The addition of 5-FU into NPs is expected to improve its pharmacokinetics, which reduces the high doses of the drug demand [21]. Hence, our aim was to investigate the anticancer activity of 5-FU loaded PLGA NPs and PCL NPs to produce 5-FU delivery systems.

The properties of the prepared systems were investigated for their particle sizes, zeta potential, drug loading capacity and *in vitro* drug release rate. In addition, these formulations were coated with C to facilitate the cellular uptake, which tested for their anti-cancer activity using the colorectal cancer (HT-29) cells.

## 2. Experimental

### 2.1. Materials

5-FU (purity > 99%), PLGA (MW 40000–75000 Da), PCL (MW 42,000 Da) and MTT (3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyltetrazolium bromide) were purchased from "Sigma-Aldrich (St. Louis, MO)". Polyvinyl alcohol (PVA) [MW 16,000 Da] and dichloromethane (DCM) were obtained from "Acros Organics (Geel, Belgium)". Human colon cancer cells (HT-29) were obtained from American type cell culture (ATCC, USA). Phosphate buffer saline (PBS pH 7.4) was selected as the release medium and prepared in the laboratory. All other reagents and chemicals were of analytical grade.

### 2.2. Preparation of 5-FU loaded NPs

5-FU loaded PLGA and PCL NPs (5-FU PLGA NPs and 5-FU PCL NPs, respectively) were prepared by using modified double emulsion (W1/O/W2) technique [22]. The composition of each formulation is presented in Table 1. Briefly, 5 mg of 5-FU was dissolved in 0.5 ml of purified water by vortex mixing. Then, 5-FU solution was emulsified in 2 ml of DCM containing dissolved amounts of polymers (40 mg) using a probe sonicator for 60 s at 60% power under ice bath. The formed primary emulsion was immediately mixed with an aqueous solution of 1% PVA (40 ml) followed by probe-sonication for 3 min. Thus, the DCM was then removed on overhead stirrer at a stirring rate of 500 rpm and room temperature for 2 h. The NPs were separated by using Centrifuge at 30,000 rpm for 15 min at 4 °C. The washing step of the precipitant was repeated more times. The supernatant solutions were then analyzed for its drug content by high performance liquid chromatography (HPLC) analysis and used to calculate entrapment efficiency (EE%) [23]. Then the precipitant was dispersed in aqueous solution by vortexing for 5 min.

C-coated NPs (C-NPs) were obtained by incubating a certain volume of the suspensions of 5-FU PLGA-NPs and FU PCL-NPs with an equivalent volume of 2 mg/ml chitosan in acetic solution (0.5%) for 2 h at room temperature [24]. The resulted C-5-FU PLGA-NPs and C-5-FU PCL-NPs were centrifuged, washed twice and then redispersed in an equivalent volume of distilled water. The all obtained NPs dispersions were stabilized using a Freeze Dryer (Alpha 1-4 LD Plus, Martin Christefriertrocknugsanlagen GmbH, Osterode am Harz, Germany) at −60 °C for 3 days. All formulations were prepared in triplicate.

### 2.3. Particle size and zeta potential measurements

The mean particle size and polydispersity index (PDI) of each formulation were measured by photon correlation spectroscopy (PCS) using a Zetasizer Nano ZS (Malvern Instruments, UK). The particle size of the prepared NPs dispersions was evaluated using dynamic light scattering (DLS) mode at the 25 °C after proper dilution. The scattering angle for measurement was set at 90°. The mean (average) particle size of each formulation was determined by taking the mean of three different readings. Zeta potential of each formulation was evaluated by laser doppler velocimetry (LDV) mode using the same Nano ZS at 25 °C. The samples were properly diluted with deionized water, sonicated and subjected for the measurement of zeta potential. All experiments were performed in triplicate. Each value reported is the average of three measurements.

### 2.4. Entrapment efficiency and drug loading

As mentioned before, the amounts of 5-FU entrapped into NPs formulations were determined indirectly by ultracentrifugation method using OptimaTM Max-E, Ultra Centrifuge (Beckman Coulter, Pasadena, CA) at 4 °C. The non-entrapped 5-FU amount in the supernatant after centrifugation was determined by HPLC method.

The drug loading (DL%) was estimated by dissolving 5 mg of freeze-dried NPs in 0.5 ml of DCM. Then, DCM was evaporated at room temperature. Then, a certain volume of deionized water was added to residue and kept in a water bath sonicator for 10 min to extract 5-FU into aqueous phase. To obtain a clear solution of 5-FU, the aqueous phase was separated by centrifuge at 5000 rpm for 10 min at 25 °C. 5-FU content was determined using HPLC [23].

Entrapment efficiency (EE%) and drug loading (DL%) were calculated according to the following equations:

$$\text{EE\%} = \frac{\text{5-FU}_{\text{total}}\text{-5-FU}_{\text{free}}}{\text{5FU}_{\text{toal}}} \times 100 \quad (1)$$

$$\text{DL\%} = \frac{\text{Amount of entrapped 5-FU}}{\text{Total weight}} \times 100 \quad (2)$$

$\text{5-FU}_{\text{total}}$ is the amount of drug added, while $\text{5-FU}_{\text{free}}$ is the free amount of drug presented in the supernatant.

### 2.5. The particle surface morphology

The particle surface morphology of the 5-FU loaded NPs was visualized by Scanning Electron Microscopy [SEM] (JSM-6360 LV, JEOL, Tokyo, Japan) technique. The freeze-dried samples were fixed on carbon tape and sputter-coated with a thin gold layer under an argon atmosphere using a gold sputter module in a high-vacuum evaporator (JFC-1100 fine coat ion sputter; JEOL, Tokyo, Japan). The coated samples were then scanned and photomicrographs were taken at an acceleration voltage of 10 kV.

### 2.6. In vitro *release study*

*In vitro* release of 5-FU loaded NPs formulations was evaluated using the dialysis membrane method as previously described [14,20]. The dialysis bag (molecular weight cut off: 5 kDa, Livingstone, NSW, Australia) was soaked in distilled water for 12 h before use. The obtained freeze-dried NPs formulations (equivalent to 5 mg of the 5-FU) were dispersed in 3 ml of PBS (pH 7.4) and put in a dialysis bag. Then, the bag was incubated with 40 ml of preheated PSB in beakers as release medium. The sink conditions for *in vitro* release studies were met since the aqueous solubility of 5-FU has been reported as approximately 10–12 mg/ml [15,16]. The beakers were placed in a thermostatic shaker at 37 °C and 100 rpm. Three ml of the aliquots was collected at predetermined time points and replenished immediately with the equal volume of fresh PBS (pH 7.4) to maintain the sink conditions. The release of free 5-FU (5-FU suspended in PBS) was performed as a control. The amount of 5-FU in the aliquots was analyzed by HPLC [23]. The experiments were done in triplicate.

**Table 1**
The composition of 5-fluorouracil (5-FU) loaded nanoparticles.

| Ingredients | 5-FU PLGA NPs | 5-FU PCL NPs | C-5-FU PLGA NPs | C-5-FU PCL NPs |
|---|---|---|---|---|
| PLGA (mg) | 40 | – | 40 | – |
| PCL (mg) | – | 40 | – | 40 |
| C (% w/v) | – | – | 2% | 2% |
| 5-FU (mg) | 5 | 5 | 5 | 5 |

PLGA: Poly lactide-co-glycolide; PCL: Poly-caprolactone; C: chitosan; 5-FU: 5-fluorouracil.

### 2.7. HPLC analysis

5-FU was determined using a reverses phase-HPLC (RP-HPLC) method reported in literature with minor modifications [23]. The HPLC system (Waters™ 600 controller, USA) equipped with wavelength detector (Waters™ 2487 a Dual λ Absorbance detector, USA), pump (Waters™ 1252 a Binary pump, USA) and an automating sampling system (Waters™ 717 Plus Autosampler, USA) was used. The HPLC system was monitored by "Empower (Waters, USA)" software. 5-FU was analyzed using mobile phase consisted of 40 mM phosphate buffer adjusted to pH 7.0 by 10% w/v potassium hydroxide. The mobile phase flowed over a reversed-phase $C_{18}$ column (Bondapak™, 4.6 × 150 mm, 10 μm particle size) at a rate of 1 ml/min. The injection volume of each 5-FU sample was 20 μl and detected by the UV detector at 260 nm. All the operations were carried out at room temperature.

### 2.8. Kinetic analysis of drug release of 5-FU

Kinetic analysis of drug release of 5-FU from different NPs was performed using various mathematical models such as "zero order", "first order", "Higuchi", "Hixon-Crowell" and "Korsemeyer-Peppas" models [25–27]. Hence, the drug release data of 5-FU from different NPs was fitted into the following models:

$$\text{"Zeroorder"} \quad Q_t = Q_0 + K_0 t \tag{3}$$

$$\text{"First order"} \quad \mathrm{Log}C = \mathrm{Log}C_0 - \frac{K_1 t}{2.303} \tag{4}$$

$$\text{"Higuchi"} \quad Q_t = kt^{0.5} \tag{5}$$

$$\text{"Hixon-Crowell"} \quad (W_0^{\frac{1}{3}} - W_t^{\frac{1}{3}}) = k_h t \tag{6}$$

$$\text{"Korsemeyer-Peppas"} \quad \frac{Q_t}{Q_\infty} = k_p t^n \tag{7}$$

"In which, $Q_0$, $Q_t$ and $Q_\omega$ are the amounts of 5-FU released initially, at time t and at time ω, respectively". "$C_0$ and C are the amounts of 5-FU initially and at time t, respectively". "$W_0$ and $W_t$ are the amounts of 5-FU in NPs initially and at time t, respectively". $K_0$, $k_1$, k, $k_h$ and $k_p$ are zero order, first order, Higuchi, Hixon-Crowell and Korsemeyer-Peppas rate constants, respectively". The exponent n (diffusion coefficient) is used to characterize the mechanism of drug release.

### 2.9. *In vitro* cytotoxicity studies

The cytotoxic effect of 5-FU loaded NPs was evaluated by testing the ability of the reducing enzymes presents in viable cells to convert MTT to formazan crystals. The cytotoxicity was performed using human colon cancer (HT-29) cells by MTT assay. Dulbecco's Modified Eagle's Medium (DMEM) was used as a major cell growth medium and a humidified atmosphere (5% $CO_2$) was maintained in cell culture. HT-29 cells were seeded in a 96-well culture plate and treated with medium at 37 ± 1 °C at $2 \times 10^4$ cells per well for 24 h. The cell medium in test wells was then changed to serum free medium (SFM) containing the tested formulations, while the cell medium in control wells was changed to serum free medium containing an equivalent volume of solvent (dimethyl sulfoxide). After incubation at 37 °C for 48 h, SFM in control and test wells were replaced by 100 μl/well of MTT solution (0.5 mg/ml) in PBS (pH 7.4) and incubated at 37 °C for another 3 h. MTT solution was removed and the purple formazan crystals formed at the bottom of the wells were dissolved using 100 μl of isopropyl alcohol/well with shaking for 1 h at room temperature.

Then, the absorbance was measured at 549 nm in a microplate reader (ELX 800; Bio-Tek Instruments, Winooski, VT, USA). The cell viability was calculated using the following equation:

$$\text{Cell viability\%} = \frac{\text{Untreated cells-Treated cell}}{\text{Untreated cells}} \times 100 \tag{8}$$

**Table 2**
The physicochemical characterization of 5-FU loaded nanoparticles.

| Formulations | Particle size (nm) | PDI | Zeta potential (mV) |
|---|---|---|---|
| 5-FU PLGA NPs | 217.5 ± 5.7 | 0.173 ± 0.003 | −17.47 ± 1.88 |
| 5-FU PCL NPs | 188.1 ± 7.4 | 0.115 ± 0.006 | −14.22 ± 0.47 |
| C-5-FU PLGA NPs | 302.2 ± 9.1 | 0.226 ± 0.019 | 15.21 ± 1.72 |
| C-5-FU PCL NPs | 271.3 ± 2.8 | 0.296 ± 0.032 | 12.08 ± 1.87 |

### 2.10. Statistical data analysis

Data analysis was carried out with the software package, Microsoft Excel, Version 2010 and origin software, version 6.1 (Northampton, Massachusetts, USA). Results are expressed as mean ± standard error (n = 3, independent samples).

## 3. Results and discussion

### 3.1. Particle size and zeta potential measurements

C-5-FU loaded different NPs ware prepared for the purpose of cellular uptake to enhance their anticancer efficiency. Thus, various 5-FU NPs depending on the polymers were prepared (Table 1). Utilization of PLGA and PCL NPs as tumor targeted delivery was employed in previous articles [28,29].

The obtained NPs were characterized in terms of particle size, PDI and zeta potential (Table 2). It was observed that all investigated NPs dispersions exhibited particle sizes in the nano-range with low PDI ( < 0.3). Results showed that the particle sizes of 5-FU NPs formulations were 217.5 ± 5.7 and 188.1 ± 7.4 nm for 5-FU PLGA NPs and 5-FU PCL NPs, respectively. The reduction of the particle size could be attributed to the high flexibility of the PCL chain compared to PLGA [30]. Moreover, the particle sizes of C-coated NPs were significantly increased. The mean particle size of C-5-FU NPs depends on the polymers used as 302.2 ± 9.1 and 217.3 ± 2.8 nm for C-5-FU PLGA NPs and C-5-FU PCL NPs, respectively. Thus, an increase of the polymer content in presence of C led to an increase of particle size of the prepared NPs [31]. Moreover, the high amounts of the polymers produced highly viscous dispersed phase leading to larger particle size of NPs [32,33]. The recorded values of PDI were less than 0.3,

**Table 3**
The yield %, entrapment efficiency (EE%) and drug loading (DL%) of 5-FU loaded nanoparticles (mean ± SD, n = 3).

| Formulations | Yield% | EE% | DL% |
|---|---|---|---|
| 5-FU PLGA NPs | 93.63 ± 4.58 | 31.69 ± 2.24 | 5.25 ± 0.15 |
| 5-FU PCL NPs | 91.32 ± 7.245 | 37.78 ± 1.65 | 7.16 ± 0.43 |
| C-5-FU PLGA NPs | 87.52 ± 4.52 | 44.05 ± 3.98 | 9.27 ± 1.41 |
| C-5-FU PCL NPs | 96.51 ± 5.67 | 51.16 ± 2.77 | 11.92 ± 3.25 |

which represent a relatively narrow particle size distribution in the all NPs (Table 2).

The zeta potential values were −17.47 ± 1.88 and −14.11 ± 0.78 mV for C-5-FU PLGA NPs and C-5-FU PCL NPs, respectively. The negative surface charge of 5-FU NPs was probably due to the presence of uncapped carboxylic groups of the polymers on the NPs surface. On the other hand, it was detected that the presence of C converted the zeta potential values to positive values as 15.21 ± 1.72 and 12.08 ± 1.78 mV for C-5-FU PLGA NPs and C-5-FU PCL NPs, respectively. This behavior was possible due to its shielding effect at the NPs surface [34]. The positive zeta potential was possible due to amine groups in the C structure, proposing that C is effectively coated onto the PLGA NPs and PCL NPs surfaces [35].

### 3.2. Entrapment efficiency (EE%) and drug loading (DL%)

Table 3 shows the effect of the studied polymers on the EE (%) and DL (%) of 5-FU loaded NPs. The EE (%) and DL (%) were found to be depending on the polymers used. An increase in EE (%) from 31% to 53% and DL (%) from 5% to 12% were recorded for 5-FU PLGA NPs and C-5-FU PCL NPs, respectively. These results revealed that the C-5-FU PCL NPs has a significant higher EE (%) and DL (%) than other NPs formulations. This could be attributed to the presence of PCL and C. This action might be explained by surface properties of C that occur after addition of this polymer throughout the preparation. The pKa value of 5-FU has been reported as 8.0 and the pH of an aqueous solution of 5-FU has been reported as 8.4 [36]. Therefore, 5-FU is negatively charged in an aqueous solution [36,37]. Chitosan is positively charged because its pKa value reach to 6.5 at suitable (acidic) pH conditions [37]. Therefore, it was attributed to the electrostatic interaction between positively charged C and negatively charged 5-FU, which lead to adsorb some of the 5-FU on to the surface of the NPs [35].

### 3.3. The particle morphology

The morphological characteristics of 5-FU loaded NPs were observed by SEM. The NPs were found to be a spherical in shape with a relatively smooth surface (Fig. 1). The part of an aggregation of NPs is probably due to drying process and their high surface energy. DLS gives a hydrodynamic diameter of NPs, while SEM gives an actual diameter of NPs in the dry state.

### 3.4. In vitro *release studies*

The release profiles of 5-FU from the NPs formulations compared to drug solution as a control is shown in Fig. 2. 5-FU was released rapidly from the dialysis bag within the first hours by using the drug solution as a control. Moreover, 5-FU loaded NPs showed a biphasic pattern with an initial burst drug release followed by sustained release profile. It was observed that the drug release is strongly influenced by the properties of polymer matrix. Moreover, 5-FU was rapidly released within the first 8 h, followed by a slow release from 8 up to 72 h. The rapid 5-FU release was mostly due to the NPs surface 5-FU, which could simply diffuse in the first 8 h (Fig. 2). The release rate of 5-FU from the NPs in the first 8 h were about 51%, 25%, 62% and 33% for C-5-FU-PCL NPs, 5-FU PCL NPs, C-5-FU-PLGA NPs and C5-FU-PLGA NPs, respectively. The slow release could be caused by the diffusion of the drug from the NPs matrix following by the degradation of the polymer. About 63%, 38%, 76% and 47% 5-FU for C-5-FU-PCL NPs, 5-FU PCL NPs, C-5-FU-PLGA NPs and C5-FU-PLGA NPs, respectively, were released after 72 h.

In general PLGA NPs showed slower drug release, which might be attributed to its high crystalline nature [7]. Consequently, PCL exhibited less water permeability, which lead to very slow degradation rate [38]. Thus, the release rate of 5-FU was increased with the addition of C to this polymer. The high release of 5-FU NPs in the presence of C might be attributed to a higher degree of water permeability by the surface of C [29]. In addition, the higher release of 5-FU was obtained from NPs containing hydrophilic glycolide units like PLGA NPs compared to a PCL NPs. In the case of PLGA NPs, the highest drug release was achieved after 72 h. It was supposed that the increasing of the hydrophilicity of PLGA improved the water permeability of the NPs layers, thereby increasing drug release [39]. Moreover, C is more hydrophilic than PLGA, which results in more hydration of NPs matrix and more drugs released during the same time [38].

### 3.5. Kinetic analysis of drug release of 5-FU

The results of *in vitro* release studies were fitted to many kinetic models. Kinetic analysis data of release of 5-FU from different NPs are presented in Table 4. The drug was released following "Higuchi square-root kinetics". "If the value of exponential n is equal to 0.5, it indicates Fickian diffusion mechanism. 'However, if the value of n is greater than 0.5 but less than 1.0, it indicates non-Ficknian diffusion mechanism'. The value of "n" in 5-FU NPs was recorded in the range of 0.503–0.529, indicating non-Ficknian diffusion mechanism [26].

### 3.6. In vitro *cytotoxicity*

We examined the effects of 5-FU-loaded NPs *versus* their corresponding bases, as well as 5-FU solution as a control on the viability of the HT-29 cells by MTT assay. The results of MTT assay are presented in Fig. 3. The results presented that both 5-FU NPs and pure 5-FU exerted anticancer activity. Particularly, 5-FU NPs enhanced the cytotoxicity of 5-FU, compared to the control after 48 h. This is a remarkable result, taking into account the slow release of 5-FU from NPs compared with the control, which is soluble in the incubation medium. All the tested 5FU NPs, as well as pure 5-FU significantly inhibited growth of HT-29 cells compared with the cells that were treated with unloaded NPs.

In addition, C-5-FU PLGA NPs and C-5-FU PCL NPs formulations enhanced 5-FU-induced growth-inhibitory activity against HT-29 cells at 48 h treatment period. These data indicate that C can enhance the anticancer activity of 5-FU as a coated layer for NPs. A maximum activity (66% loss of cell viability) for C-5-FU PLGA NPs was noted with HT29 cells over a 48 h incubation period.

The enhanced effect of these NPs could be attributed to the strong electrostatic interactions between positively charged C and the negative glycocalyx on the cell membrane [40]. This effect increased NPs-cell membrane interactions, which facilitate the anticancer activity.

## 4. Conclusion

In this study, 5-U loaded C-coated PLGA and PCL NPs have been successfully formulated as nano-carriers delivery system for cancer therapy. 5-FU loaded NPs exhibited particle sizes with nano-range, spherical-shape, reasonable EE (%), DL (%) and sustained release patterns of 5-FU. 5-FU loaded C-PCL NPs with desired EE (51%).

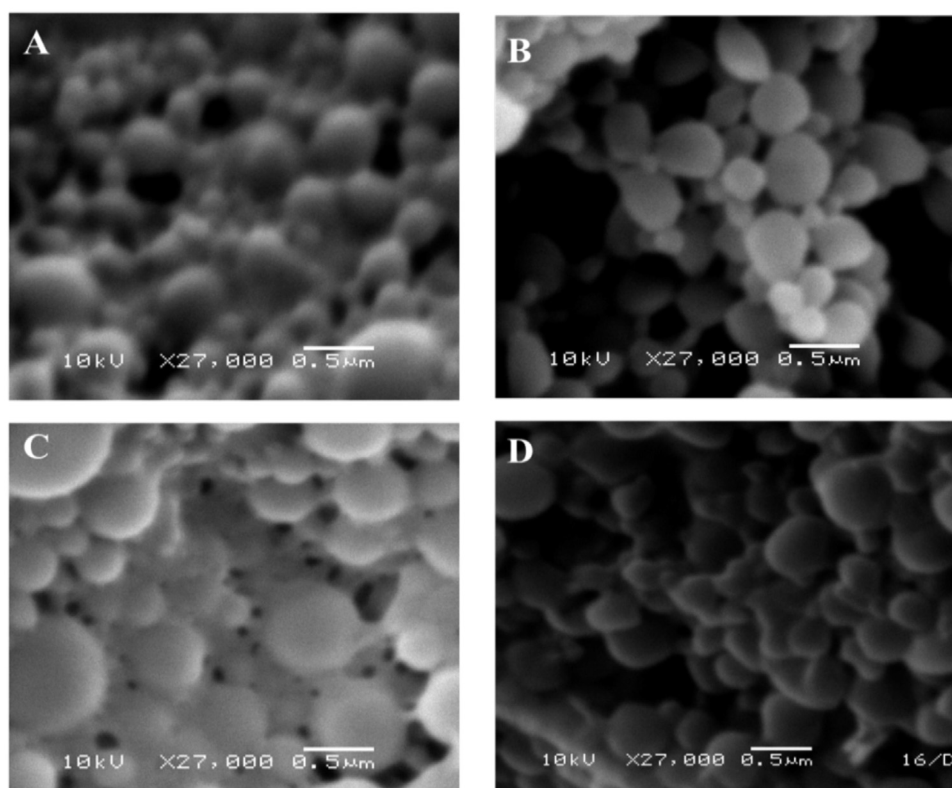


**Fig. 1.** SEM micrographs of 5-FU loaded nanoparticles, (A) 5-PLGA NPs; (B) 5-CL NPs; (C) C-5-PLGA NPs; (B) C-5-PCL NPs.

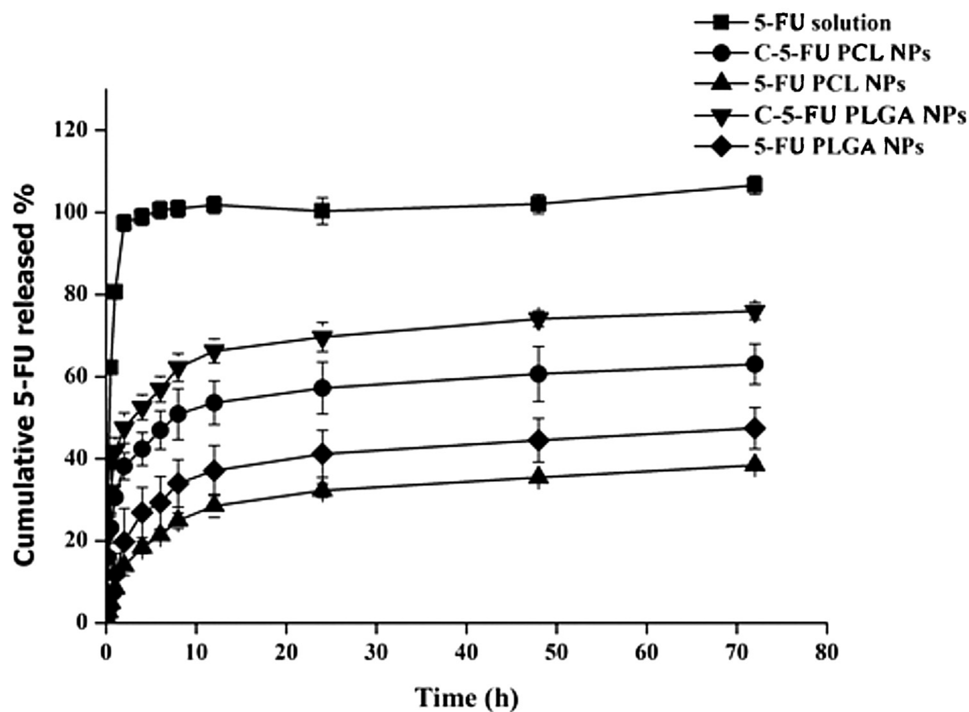


**Fig. 2.** Dissolution profiles of 5-FU solution (control) and 5-FU loaded NPs in phosphate buffer pH 7.4 (mean ± SD, n = 3).

**Table 4**
Release kinetics of 5-FU loaded NPs using different kinetic equations.

| Codes | $R^2$ (Zero order) | $R^2$ (First order) | $R^2$ (Higuchi) | $R^2$ (Korsmeyer-Peppas) | "n" value |
|---|---|---|---|---|---|
| 5-FU PLGA NPs | 0.758 | 0.804 | 0.905 | 0.939 | 0.503 |
| 5-FU PCL NPs | 0.772 | 0.802 | 0.914 | 0.926 | 0.511 |
| C-5-FU PLGA NPs | 0.781 | 0.864 | 0.925 | 0.948 | 0.529 |
| C-5-FU PCL NPs | 0.740 | 0.809 | 0.893 | 0.899 | 0.525 |

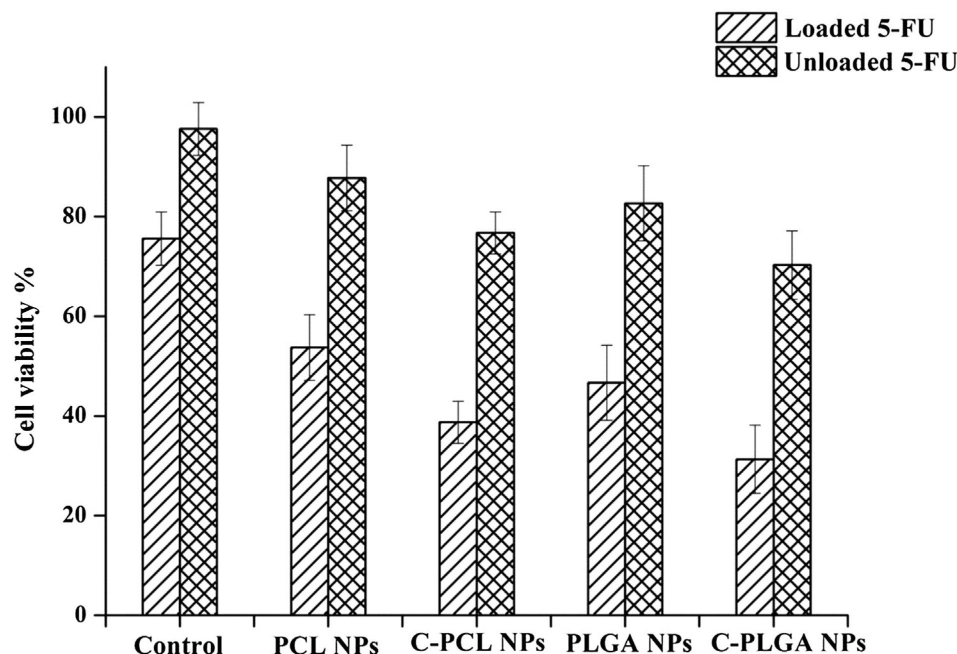


**Fig. 3.** Percent cell viability of HepG2 treated with a free 5-FU solution, unloaded NPs and 5-FU loaded NPs after incubation for 48 h, (mean ± SD, n = 3).

*In vitro* release studies showed that NPs exhibited a sustained release behavior. The *in vitro* cytotoxicity studies on HT-29 cells showed that 5-FU loaded C-coated NPs have a greater effect than 5-FU solution and unloaded NPs. Based on these results; 5-FU loaded C-coated NPs can be considered as a promising carrier for cancer therapy.

## Competing interests

The authors declare that they have no competing interests associated with this manuscript.

## Acknowledgements

This Project was funded by the National Plan for Science, Technology and Innovation (MAAREFAH), King Abdulaziz City for Science and Technology, Kingdom Saudi Arabia, Award No. 11-NAN1461-02.

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