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# Development of chitosan nanoparticles as drug delivery systems for 5-fluorouracil and leucovorin blends

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ABSTRACT

Both 5-fluorouracil (5-FU) and leucovorin (LV) are hydrophilic drugs which are used in combination for the treatment of colon cancer. Chitosan (CS) nanoparticles were prepared by ionic gelation technology, and then used for trapping 5-FU and LV. Combination drugs were encapsulated into CS nanoparticles as a result of electrostatic interactions, which was confirmed by Fourier transform spectroscopy (FTIR). XRD results demonstrated that the both drugs were distributed in the CS nanoparticles in the amorphous state. Efficient encapsulation efficiency (EE) and loading capacity (LC) were achieved which were correlated to their initial drug concentration. Simultaneous release of 5-FU and LV from CS nanoparticles were observed *in vitro* studies; both 5-FU and LV experienced initial burst release which was followed by a constant and continuous release. The release of drugs was influenced by their initial drug concentration, indicating that the release of drugs could be controlled by varying the initial drug concentration. All results suggested that CS nanoparticles are promising system for simultaneously delivering 5-FU and LV in treatment of colon cancer.



## 1. Introduction

The discovery of new drug delivery systems can extend the potential of traditional anticancer agents in the treatment of cancer diseases. Polymer-drug conjugates (nano- or micro-particles) are well established for the delivery of a single anticancer agent, and improved therapy of traditional drugs can be achieved for traditional drugs in cancer treatments (Greco & Vicent, 2009). However, the administration of different drug loaded particles is required to achieve the therapeutic effect resulting from combinations of two different drugs. Therefore, development of combination drug loaded delivery systems is crucial due to their promising therapeutic potential in the treatment of various diseases.

Combination therapy is attracting growing interest due to its improved therapeutical efficiency against many diseases, such as the arterial disease (Wan, Sun, Gao, & Li, 2009), malignant tumors (Kaplan, Mcconnell, Roehrborn, Meehan, et al., 2006), osteoporosis (Pinkerton & Dalkin, 2007), type 2 diabetes (Feinglos, Thacker, Lobaugh, Deatkine, et al., 1998) and hypertension (Moser & Black, 1998). It would be beneficial to develop a drug delivery system that could carry more than one therapeutic agent (Hammady, El-Gindy, Lejmi, Dhanikula, et al., 2009); however, most of the drug delivery systems are commonly applied for the delivery of single therapeutic agent (Greco & Vicent, 2009). Due to the molecular complexity of cancer diseases, different therapy types (radiation and chemotherapy) and simultaneous administration of two or more different types of drugs are generally necessary (Greco & Vicent, 2009). Although the standard administration of 5-FU in combination with LV is well acknowledged in the treatment of colorectal cancer, only a few approaches consider the combination use of 5-FU and LV in developing colon specific drug delivery systems (Lamprecht, Yamamoto, Takeuchi, & Kawashima, 2005).

Compatibility between hydrophobic polymer and hydrophilic drugs has been a major challenge for efficient drug entrapment of multiple hydrophilic drugs. Most drugs were mainly attached on the surface of particles instead of uniformly distributed in the microparticle matrix due to the incompatibility of hydrophobic and hydrophilic phases. It was thought that the separation of drugs from the particles was caused by the lack of dynamic force which is required for hydrophilic drug to migrate into the inner part of the particles during the preparation process. Most of the drugs attached on the surface would desorb during the preparation and purging process. Lamprecht et al. (2005) designed and prepared microspheres co-loaded with 5-FU and leucovorin using a hydrophobic polymer; however, no successful drug entrapment and subsequent release was achieved. The application of hydrosoluble polymer provides a novel approach to circumvent this problem.

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**Table 1**
Formulation of CS nanoparticles loaded with combination drugs.

| Formulation code | 5-FU concentration (mg/mL) | LV concentration (mg/mL) |
|---|---|---|
| F1 | 5 | 2 |
| F2 | 7.5 | 2 |
| F3 | 10 | 2 |
| F4 | 5 | 4 |
| F5 | 5 | 6 |

Chitosan (CS) is an abundant polysaccharide present in nature, which is comes closely second to cellulose. It can be obtained by partial deacetylation of chitin in alkaline solution (Subramanian, Rau, & Kaligotla, 2006). CS is attracting more and more attention in the application as a drug delivery carrier due to its intrinsic nature, such as biodegradability, biocompatibility, nontoxicity, nonimmunogenic, noncarcinogenic, and antibacterial properties (Sun & Wan, 2007, Tiyaboonchai, 2003). In addition, CS is positively charged and exhibits absorption enhancing effects (Ravi Kumar, 2000). The most important feature of CS is that it is soluble in most organic acidic solution at pH less than 6.5 and can easily form nanoparticles by crosslinking with agents such as glutaraldehyde (Chen, Wu, Mi, Lin, et al., 2004; Lin, Chen, & Luo, 2007), tripolyphosphate (Mi, Shyu, Chen, & Lai, 2002) and polyaspartic acid sodium salt (Zheng, Yang, Wang, Hu, et al., 2007). Therefore, CS is an ideal drug delivery carrier for hydrophilic drugs such as 5-fluorouracil and leucovorin.

Based on this rational, a combination drug delivery system co-loaded with 5-fluorouracil and leucovorin was prepared by using multifunctional CS. These combination drug-loaded delivery systems offer some interesting features: (1) the preparation of the combination drug-loaded system can be formed in aqueous solution without the use of organic solvent, surfactant, and high shearing force; (2) the CS nanoparticles are positively charged, therefore can improve the site-specific targeting due to the strong affinity towards negatively charged biological membranes; (3) the components are made from water soluble CS and the hydrophilic drugs can be effectively encapsulated into the nanoparticles; and (4) two drugs were encapsulated into one drug delivery system, which provided a standard therapy for colon cancer treatment.

## 2. Materials and methods

### 2.1. Materials

Chitosan (CS) ($M_w$ 100,000, deacetylation degree 85%), derived from crab shell, was purchased from Zhanjiang Xingmao Chemical-Glass Co. Ltd. (Zhanjiang, China). 5-Fluorouracil (5-FU), leucovorin (LV), and sodium tripolyphosphate (TPP) were sourced from Aldrich Chemical Company Inc. (Sydney, Australia). All other chemicals were of analytical grade and obtained from the chemical store of Deakin University (Geelong Campus, Australia).

### 2.2. Preparation of CS nanoparticles

The preparation of CS nanoparticles is based on an ionic interaction between positively charged CS solution and negatively charged TPP solution (Li, Wang, Chen, Zeng, et al., 2011). Fig. 1 displays the schematics of CS nanoparticle preparation procedures. CS was firstly dissolved in 1% aqueous acetic acid solution at a concentration of 3 mg/mL, and TPP was dissolved in distilled water with a concentration of 2 mg/mL. 5-FU and LV were dissolved directly in TPP solution at different concentrations (Table 1) before the synthesis of CS nanoparticles. Then, 4 mL TPP solution (2 mg/mL) containing combination drugs was dropped into 10 mL CS solution under magnetic stirring (1000 rpm) at room temperature. CS

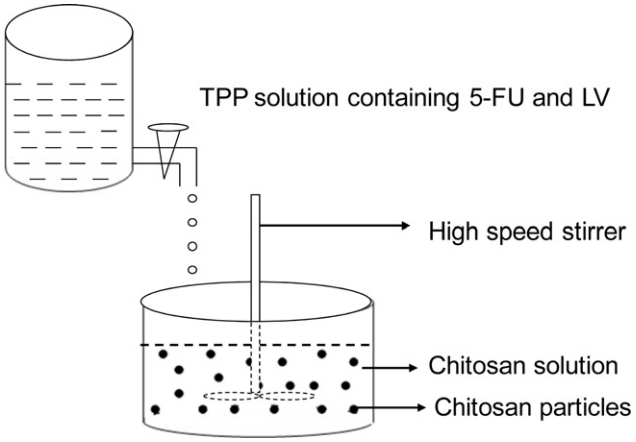


**Fig. 1.** Scheme for the preparation of CS nanoparticles.

nanoparticles were formed instantaneously. CS nanoparticle suspension was kept stirring for 30 min for further crosslinking of nanoparticles. Finally, CS nanoparticles were collected by centrifugation at 15,000 rpm and freeze-drying at −40 for 24 h.

### 2.3. Morphological analysis of CS nanoparticles

Transmission electron microscopy (TEM) was used in the morphological analysis of CS nanoparticles. Samples were prepared by casting one drop of freshly made nano-suspension onto copper grid with carbon film support and extra solution was removed by filter paper. Samples were observed directly with TEM (JEM-100, JEOL, Tokyo, Japan) after they were dried under room temperature without further staining.

### 2.4. FTIR analysis

CS nanoparticle suspension was centrifuged at 12,000 rpm for 20 min, the supernatant was discarded and the sediment was collected and lyophilized at −40 °C for 24 h. The FTIR spectra of samples were measured using a Fourier transform infrared spectrometer (Spectrum GX-1, PerkinElmer, USA). Briefly, a small quantity of CS nanoparticles was mixed with 200 mg KBr and compressed to form tablets. These tablets were scanned, in transmission model, in the spectral region of 4000–400 $cm^{-1}$, using a resolution of 4 $cm^{-1}$ and 32 co-added scans.

### 2.5. XRD analysis

The physical state of 5-FU and LV in the CS nanoparticle matrix was assessed by XRD studies. X-ray powder diffraction spectra of pure CS, drug free CS-TPP nanoparticles, and combination drug loaded CS nanoparticles were obtained at room temperature using X-ray diffractometer (D8 Advance, Bruck, Germany) with Cu as a target at a voltage of 40 kV. The samples were analysed in $2\theta$ angle range of 3–60° at a scanning rate of 3°/$2\theta$/min.

#### 2.5.1. Particle size and zeta potential measurement

Average particle size and zeta potential of CS nanoparticles were measured by photon correlation spectroscopy (PCS) and laser Doppler anemometry (LDA), respectively, using a Nano-ZS (Malvern Instruments, UK).

### 2.6. Evaluation of drug loading capacity and encapsulation

CS nanoparticle suspensions were separated with a centrifuge at 12,000 rpm for 20 min, and the drug encapsulation efficiency (EE)

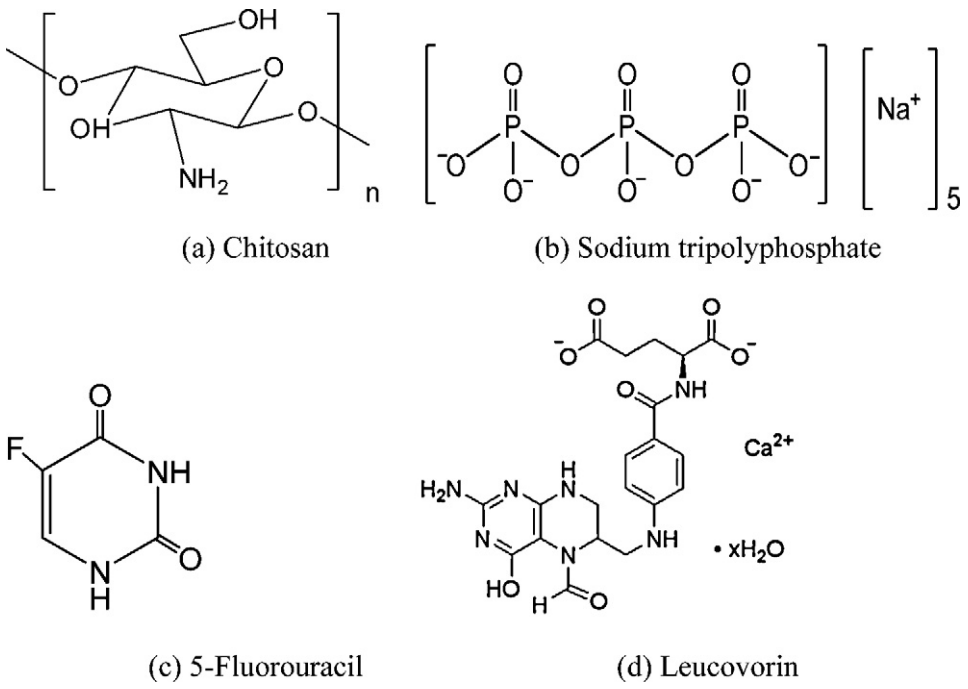


**Fig. 2.** Chemical structure of (a) chitosan; (b) sodium tripolyphosphate; (c) 5-fluorouracil; and (d) leucovorin.

and loading capacity (LC) of CS nanoparticles were evaluated by measuring the ultraviolet absorption of the supernatant. The corresponding calibration curves were made by testing the supernatant of blank CS nanoparticles. Each sample was measured in triplicate. 5-FU was measured at 266 nm where an intense characteristic peak was displayed while LV showed a characteristic peak at 288 nm.

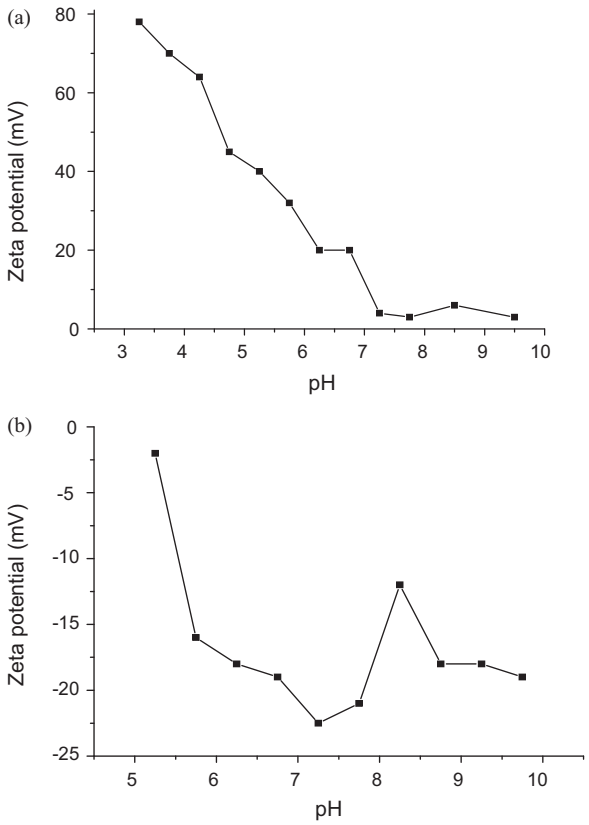


**Fig. 3.** Isoelectric titration graphs of (a) CS (3 mg/mL); and (b) TPP (2 mg/mL).

The encapsulation efficiency and loading capacity of 5-FU and LV of the CS nanoparticles were calculated according to the following equations (Papadimitriou, Bikiaris, Avgoustakis, Karavas, et al., 2008):

$$\mathrm{EE} = \frac{W_t - W_f}{W_t} \quad (1)$$

$$\mathrm{LC} = \frac{W_t - W_f}{W_n} \quad (2)$$

where $W_t$ represents the total amount of 5-FU and LV; $W_f$ is the amount of free 5-FU and LV in the supernatant; and $W_n$ is the weight of nanoparticles after freeze-drying. All measurements were performed in triplicate and the mean value was reported.

### 2.7. Evaluation of in vitro drug release

The *in vitro* drug release profile of CS nanoparticles was determined by using dialysis membrane bag. Briefly, freeze-dried CS nanoparticles (20 mg) were placed into dialysis bag (with a molecular cut-off of 5 kDa). The CS nanoparticle loaded dialysis bag was incubated in 70 mL simulated fluids. The system was maintained at 37 ± 0.5 °C with mild magnetic stirring. At appropriate

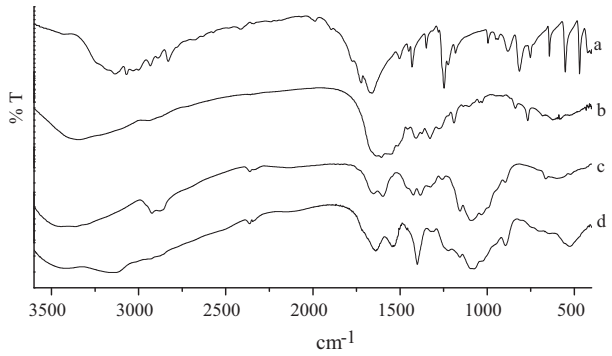


**Fig. 4.** FTIR spectra of (a) 5-FU; (b) LV; (c) CS; and (d) NPs co-loaded with 5-FU and LV.

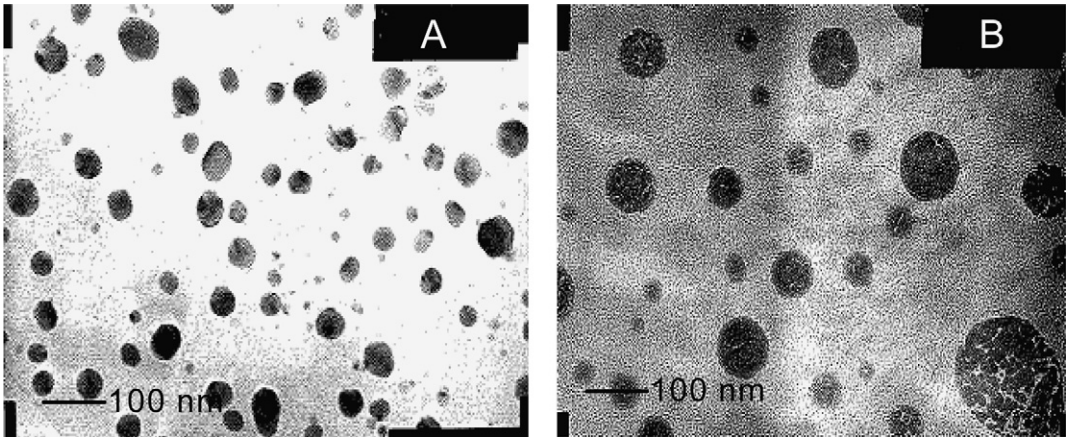


**Fig. 5.** TEM images of CS nanoparticles: (a) CS nanoparticles; and (b) combination drug loaded nanoparticles.

time interval, 4 mL of the release media was taken and equivalent volume of fresh phosphate buffered saline (PBS) solution was supplemented in order to keep the volume of the system identical. The cumulative release of 5-FU and LV was measured with ultraviolet spectrophotometer Lambda 35 (Perkin-Elmer, USA) at 266 and 288 nm, respectively.

## 3. Results and discussion

### 3.1. Formation of combination drug loaded CS nanoparticles

The preparation of CS nanoparticles was based on the ionic interaction of a positively charged CS solution (Fig. 2A) and negatively charged TPP solution (Fig. 2B) (Calvo, Remunanlopez, Vilajato, & Alonso, 1997). The charge density of both CS and TPP solution has a great effect on the ionic interaction. It is well known that the charge density is greatly influenced by the pH value of solution. In order to find an optimal pH value for the ionic interaction, an automatic isoelectric titration instrument (Nano-ZS & Multi Purpose Titrator, Malvern, USA) was used to investigate the relationship between the pH value and the charge density (zeta potential) of CS and TPP solution.

The selection of optimum charge density is a critical step for effective ionic interactions. The zeta potential of CS solution decreases gradually with the increasing pH (Fig. 3). The zeta potential reaches almost 80 mV when the pH of CS is about 3.2, and drops to about 2 mV when the pH of CS is 9.5. In the case of TPP solution, the pH value drops dramatically from −2 to −22.5 mV when its pH increases from 5.25 to 7.25. By considering the influence of pH on both CS and TPP solution, the pH range of 5.5–6.5 was chosen for the formulation of combination drug-loaded nanoparticles in this work because a relatively high absolute zeta potential was obtained for both TPP and CS solution in this range, which was favoured by the crosslinking of the oppositely charged solution.

### 3.2. FTIR analysis

Fig. 4 shows the FTIR spectra of pure 5-FU, LV, CS and CS nanoparticles co-loaded with 5-FU and LV. As shown in Fig. 4a, the intense characteristic peaks at 3160, 1727, 1662, 1426, 1247, 811.7 and 547 $cm^{-1}$ are detected due to the vibration of imide stretch (amide II and amide III) and aromatic ring in the structure of 5-FU (Fig. 2a). In the case of the FTIR spectra of LV (Fig. 4b), some absorption bands are observed at 1609, 1324, 1190, and 763 $cm^{-1}$ corresponding to the vibration of NH stretch, aromatic ring, C=O, C–H groups. The intense peaks at 1654 and 1597 $cm^{-1}$ confirmed the presence of amide I and amide II in the chemical structure of CS (Fig. 4c). After ionic crosslinking with mixture of TPP and drugs, the characteristic peak at 1654 $cm^{-1}$ in the CS spectra shifted to 1638 $cm^{-1}$, while the peaks at 1597 $cm^{-1}$ disappeared (Fig. 4d), due to the strong ionic interaction between positively charged CS and TPP solution. In addition, most of the intense characteristic peaks of both 5-FU and LV are not observed at the same position in the drug loaded nanoparticles, indicating the intense interaction between the drugs and CS.

### 3.3. Morphology of nanoparticles

Spherical nanoparticles were obtained for both drug free and drug loaded samples and the encapsulation of 5-FU and LV slightly increased the diameter of the nanoparticles as shown from the TEM images of drug free and CS nanoparticles co-loaded with 5-FU and LV (Fig. 5) and the statistical data on particle size and size distribution (Table 2). The drug free CS nanoparticles are roughly spherical in shape with a size distribution range of 15–62 nm in diameter and the mean diameter of the particles is about 35 nm (Fig. 5a and Table 2). Compared to what was observed with the drug free CS nanoparticles, significant difference can be noticed in the size of drug loaded particles (Fig. 5b). The statistical diameter of the combination drug loaded CS nanoparticles is in the range of 34–112 nm with a mean diameter of 60 nm (Table 2). This is because the loading of drug can influence the ionic interaction between TPP and polymer. As both drugs are hydrophilic and can protonate in aqueous solution, the presence of drug anion can occupy the cation of the CS and therefore influence the interaction between CS and TPP.

### 3.4. XRD analysis

X-ray diffractometry is widely employed for the investigation of the physical state of drug in the polymer matrix, as it provides some useful information on drug release mechanism and dynamics. Some drugs are dispersed in the particles in amorphous or solid solution state, while others are loaded in crystalline phase depending on drug's property and the interaction between drugs and polymers.

The investigation of physical state of drugs in the CS nanoparticle matrix with XRD has found that the drugs are distributed in solid solution state in the nanoparticles as presented from the diffraction

**Table 2**
Statistical data of particle size and size distribution.

| No. | Minimum size (nm) | Maximum size (nm) | Mean size (nm) |
|---|---|---|---|
| A | 15 | 62 | 35 |
| B | 34 | 112 | 60 |

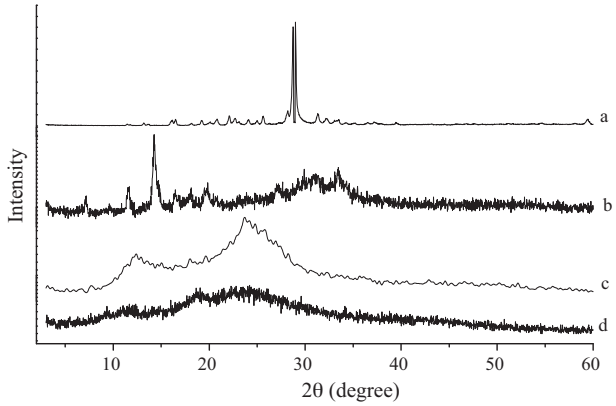


**Fig. 6.** X-ray diffraction pattern of (a) pure 5-FU powder; (b) LV; (c) CS; and (d) combination drug-loaded CS NPs.

patterns of pure 5-FU, LV, CS powder and drug loaded CS nanoparticles (Fig. 6). Intense diffraction peaks are observed at $2\theta = 16.5, 19.3, 20.7, 22.1, 28.7, 31.48, 32.2, 33.5, 59.4^\circ$ in the diffraction pattern of pure 5-FU (Fig. 6a), indicating that 5-FU exists in a crystalline powder state. The diffractogram of LV exhibited one intense diffraction peaks at $2\theta = 14.3^\circ$ and several weak diffraction peaks at $2\theta = 11.6, 19.8^\circ$ due to its crystalline properties (Fig. 6b). Intense diffraction peaks at $2\theta = 12.4$ and $23.6^\circ$ were also observed at the diffraction pattern of CS (Fig. 6c).

Although intense peaks were observed in both polymer and drugs, only broad diffraction peaks are observed in the combination drug loaded CS nanoparticles (Fig. 6d), because of the unique preparation process introduced in the current work. The preparation of drug loaded CS nanoparticles was based on the ionic crosslinking of positive charged CS and negatively charged TPP. 5-FU and LV was added into TPP solution before it was dropped into the CS solution. Two ionic interactions exist in the reaction process: an intense electrostatic interaction between the positive charged amino group of CS and the negative anion of TPP solution; the electrostatic interactions between the positive charged amino groups of CS and two oppositely charged drugs. All those ionic interactions contribute significantly to the crosslinking and subsequent formation of drug loaded CS nanoparticles, which restrain the movement of the molecular chain of both polymers and drugs. Therefore, drugs are observed existing in amorphous states in the polymer particles. A similar result was also found when pramipexole was encapsulated into CS nanoparticles (Papadimitriou et al., 2008).

### 3.5. Encapsulation efficiency and loading capacity of nanoparticles

The initial concentration of both 5-FU and LV plays an important role in the EE and LC of CS nanoparticles (Table 3). When the concentration of LV is maintained unchanged, the EE of 5-FU decreases while the LC of 5-FU increases with an increasing concentration of 5-FU. However, the increase in 5-FU concentration leads to a decrease of both EE and LC of LV. When 5-FU initial concentration is fixed, the EE and LC of LV increase from 33.39% and 9.74% to 43.09% and 23.93%, respectively, while the EE and LC of 5-FU decrease from 20.92% and 12.28% to 7.79% and 4.31%, respectively, when the initial concentration of LV increased from 2 to 6 mg/mL. It was found that the EE and LC of LV are higher than that of 5-FU when the initial concentration of 5-FU and LV is the same (5 mg/mL) (data are not provided), which could be attributed to the fact that LV is more hydrophilic than 5-FU (Lamprecht et al., 2005) and is more likely to interact with CS in aqueous medium.

**Table 3**
Encapsulation efficiency (EE) and loading capacity (LC) of CS nanoparticles.

| Formulation code | EE (%) | | LC (%) | |
|---|---|---|---|---|
| | 5-FU | LV | 5-FU | LV |
| F1 | 20.92 | 33.39 | 12.28 | 9.74 |
| F2 | 17.81 | 18.29 | 15.48 | 5.31 |
| F3 | 15.23 | 15.77 | 17.34 | 4.51 |
| F4 | 18.89 | 40.83 | 10.48 | 17.23 |
| F5 | 7.79 | 43.09 | 4.31 | 23.93 |

**Table 4**
Zeta potential and average particle size of combination drug loaded CS nanoparticles.

| Formulation code | Zeta potential (mV) | Particle size (nm) |
|---|---|---|
| F1 | 28.9 | 40.73 |
| F2 | 28.8 | 63.24 |
| F3 | 26.9 | 97.40 |
| F4 | 27.9 | 74.65 |
| F5 | 25.6 | 78.53 |

### 3.6. Zeta potential of combination drug loaded CS nanoparticles

Zeta potential and particle size are two important characteristics of nanoparticles; the stability of nanoparticles in aqueous is strongly correlated to its zeta potential. The higher the absolute zeta potential the more stable the nanoparticles due to the stronger repellent interaction between each other. In addition, it was reported that the charge density of nanoparticles plays an important role in its binding with negatively charged cancer cell membrane (Sun & Wan, 2007). Therefore, positive charged nanoparticles are ideal options for the preparation of drug delivery systems in the therapy of cancer related diseases.

Zeta potential decreases with the increase of 5-FU concentration, while the particle size of the combination drug loaded nanoparticles increases with the 5-FU loading. The influence of the initial drug concentration on the zeta potential and particles size of the combination drug loaded nanoparticles is compared in Table 4. When the initial concentration of 5-FU increases from 5 to 10 mg/mL, the zeta potential of the nanoparticles drops from 28.9 to 26.9 mV, while the particle size increases dramatically from 40.73 to 97.4 nm. A similar phenomenon is also observed with the increasing concentration of LV when the 5-FU initial concentration maintains the same.

As indicated previously, the preparation of combination drug loaded nanoparticles is based on two groups of ionic interaction of oppositely charged solution: (1) strong ionic interaction between amino group of CS and anion group of TPP solution; (2) the weak ionic interaction between amino groups of CS and 5-FU and the weak ionic interaction between amino groups of CS and LV. Therefore, the increase of both 5-FU and LV can reduce the interaction between CS and TPP. In addition, dissolving both drugs into the TPP solution can lead to the decrease of pH value of TPP solution and lower the charge density of TPP solution. Consequently the structures of the combination drug loaded nanoparticles become less compact.

### 3.7. In vitro release of 5-FU and LV from CS nanoparticles

The *in vitro* release profile of the combination drug loaded CS nanoparticles was investigated by using dialysis method. The release of both drugs from the CS nanoparticle experienced an initial burst release which followed by a constant and continuous release (Fig. 7). The initial burst drug release is due to desorption and diffusion of drugs from the surface of the combination drug loaded nanoparticles. Following the incubation in simulated gastrointestinal fluids the drug loaded CS nanoparticles undergo

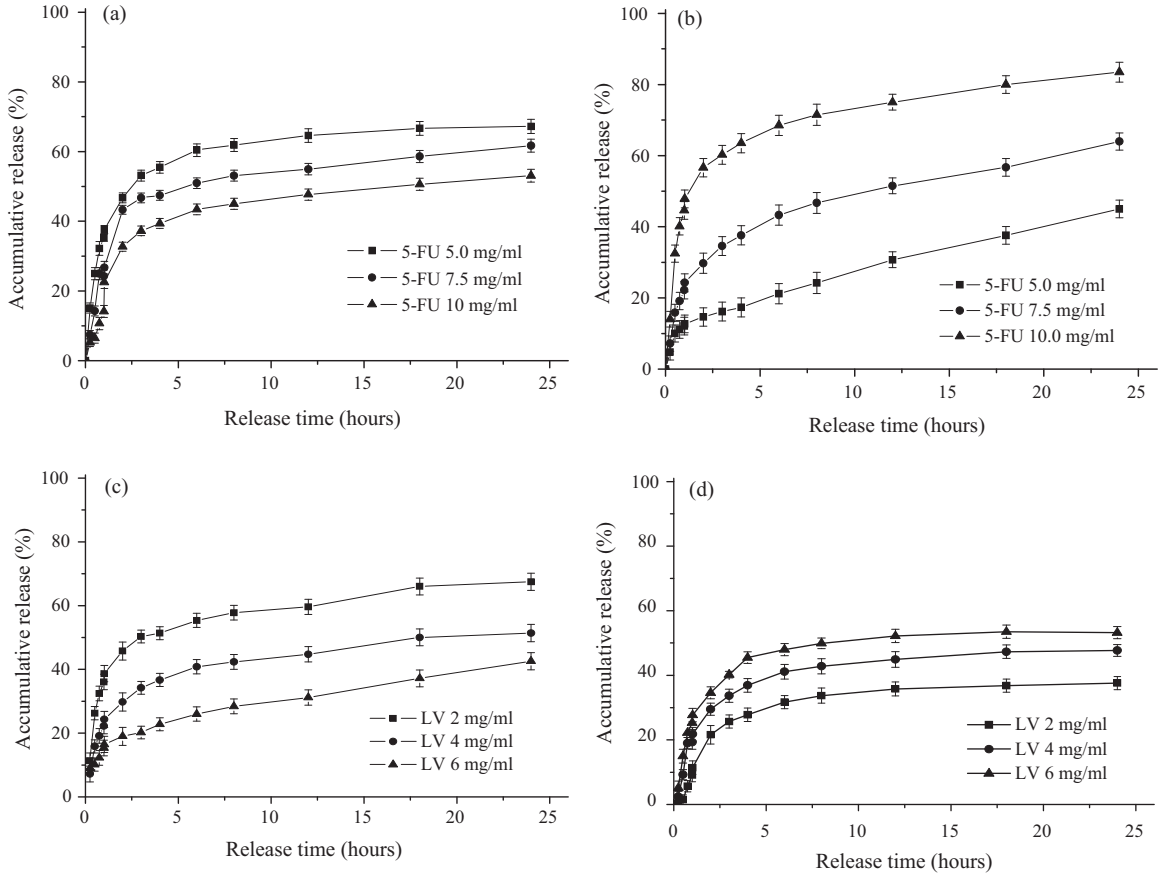


**Fig. 7.** The effects of 5-FU and LV initial concentration on the two-drug release patterns: (a) 5-FU on 5-FU; (b) 5-FU on LV; (c) LV on LV; and (d) LV on 5-FU.

gradual swelling, leading to the constant and slow release of drugs from CS nanoparticles.

It is obvious that the release of the two drugs is significantly affected by the initial concentration of both drugs. Fig. 7a and b shows the influence of the initial concentration of 5-FU on the release of 5-FU and LV. The higher the initial concentration of 5-FU, the slower the release of 5-FU from the combination drug loaded CS nanoparticles. For instance, the cumulative release of 5-FU is 55%, 46% and 39% for the nanoparticles with 5-FU initial concentration of 5 mg/mL, 7.5 mg/mL and 10 mg/mL after 4 h incubation, and reaches 66.8%, 62%, and 52.6%, respectively, by the end of 24 h incubation. This can be explained from the particle size and zeta potential of nanoparticles. The increase of 5-FU concentration leads to larger nanoparticles and a decrease in zeta potential, indicating a strong interaction between drugs and polymer matrix. Drug loaded nanoparticles with low initial drug concentration are smaller, and therefore, the drug release is faster due to a larger surface area to volume ratio. In addition, when compared with smaller nanoparticles more time is required for the drugs to diffuse out from the interior of larger nanoparticles, which consequently leads to the slower release of 5-FU. A similar drug release trend was also observed from the chitosan nanoparticles loaded with BSA by Gan et al. (Gan & Wang, 2007).

In contrast to what was observed with the release of 5-FU, the cumulative release of LV from CS nanoparticles increases with 5-FU concentration. As indicated in Fig. 7b, the cumulative release of LV is 63%, 37.5% and 17.5% when the initial concentration of 5-FU is 5 mg/mL, 7.5 mg/mL and 10 mg/mL by the end of 4 h incubation and increases to 83.4%, 63.8% and 44.7% by the end of the 24 h incubation. This is because both drugs were dissolved in the TPP solution before dropping into the CS solution when the combination drug loaded nanoparticles were formulated. An increase in 5-FU concentration could restrain the interaction between LV and polymer matrix, therefore LV is mainly encapsulated in the CS nanoparticles through physical bonding. As a consequence, LV releases faster when incubated in imulated gastrointestinal fluids.

A similar phenomenon was also observed by varying the initial concentration of LV when the initial concentration of 5-FU maintained the same (Fig. 7c and d). It should point out that after 24 h incubation the maximum release rates are less than 85% due to the stability of CS nanoparticles in PBS solution. The release of drugs from the nanoparticles is based on swelling process instead of degradation or erosion. It is expected that 100% release of drug could be obtained in the presence of enzyme in the colon after 4–5 h. Furthermore, the release profile indicates that the cumulative release of drug can be modulated by varying the initial concentration and the ratio of drugs. By comparing the release of 5-FU from the single or combination drug loaded CS nanoparticles no significant difference of release pattern was observed (Yang & Hon, 2009).

## 4. Conclusions

In this work, CS nanoparticles co-loaded with 5-fU and LV were successfully fabricated by ionic gelation. Efficient drug encapsulation efficiency and loading capacity was obtained due to the strong interaction between polymer and drugs. Both drugs were found dispersing in CS nanoparticles in amorphous state, which was confirmed from the XRD results. The initial amount of the two drugs had great effect on the drug loading and encapsulation efficiency, zeta potential and particle size of CS nanoparticles. Cumulative 5-FU release decreases with the increasing initial 5-FU concentration

and decrease of LV initial concentration. A similar release pattern is also observed with the cumulative release of LV, indicating the release profile of drugs can be modulated by varying the preparation parameters. The novel properties of the combination drug loaded CS nanoparticles indicate that they are promising vehicle for effective delivery of multiple anticancer reagents in the chemotherapy of colon cancer.

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