# Study on Colon-Specific 5-Fu pH-Enzyme Di-Dependent Chitosan Microspheres

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**Colon-specific drug delivery systems (CDDS) can improve the bioavailability of drug through the oral route. A novel formulation for oral administration using pH-enzyme Di-dependent chitosan mcirospheres (MS) and 5-Fu as a model drug has been investigated for colon-specific drug delivery by the emulsification/chemical cross-linking and coating technique, respectively. The influence of polymer concentration, ratio of drug to polymer, the amount of crosslinking agent and the stirring speed on the encapsulation efficiency, particle size in microspheres were evaluated. The best formulation was optimized by an orthogonal design. Drug release studies under conditions mimicking stomach to colon transit have shown that the drug was protected from being released in the physiological environment of the stomach and small intestine. The plasma concentrations of 5-Fu after oral administration of coated chitosan MS to rats were determined and compared with that of 5-Fu solution. The *in vivo* pharmacokinetics study of 5-Fu loaded pH-enzyme Di-dependent chitosan MS showed sustained plasma 5-Fu concentration–time profile. The *in vitro* release correlated well with the pharmacokinetics profile. The results clearly demonstrated that the pH-enzyme Di-dependent chitosan MS is potential system for colon-specific drug delivery of 5-Fu.**

**Key words** 5-Fu; emulsification/chemical cross-linking; microsphere; colon specific drug delivery

Colorectal cancer is one of the most frequent causes of cancer deaths. In the United States of America (U.S.A.) more than 100000 patients develop (per year) this disease and almost half of them will die from their cancer.[1,2] For several decades, 5-Fu was the only chemotherapeutic agent with clinical activity against colon cancer.[3] Due to erratic oral bioavailability, intravenous administration of this drug is currently in clinical use.[4] However, on intravenous administration, 5-Fu rapidly distributed and eliminated with an apparent terminal half-life of 8—20 min and produces severe systemic toxic effects of gastrointestinal, hematological, neural, cardiac and dermatological origin. Most of these systemic side effects are due to the cytotoxic effect of 5-Fu after it reaches unwanted sites.[5] Targeted delivery of 5-Fu not only reduces systemic side-effects, but also provides an effective and safe therapy for colon cancer with reduced dose and reduced duration of therapy. Therefore, to obtain a long-term and constant therapeutic effect, an oral colon-specific drug delivery system (CDDS) is needed.

The different approaches for targeting orally administered drugs to the colon include coating with pH-dependent polymers, design of timed-release dosage forms and the utilization of carriers that are degraded exclusively by colonic bacteria. The poor site-specificity of pH-dependent system, because of large variation in the pH of the gastrointestinal tract, has been very well established. The site-specificity of timed-release dosage forms is considered poor because of large variations in gastric emptying times and in passage across the ileocaecal junction. The pH-enzyme Di-dependent chitosan microspheres (MS) combine the advantages of above three methods and can be produced on a large industrial scale, with low toxicity potential for other organs, produce sustained release, and can colon-specific target after oral administration.

Chitosan is a partially deacetylated polysaccharide obtained by alkaline treatment of chitin, one of the most abundant biopolymers in nature. Chitosan has been widely researched for biomedical applications such as wound healing, drug delivery systems, coatings and tissue engineering, as well as applications in food, cosmetics and agricultural industries.[6—10] Chitosan has been gaining increasing importance in the pharmaceutical field owing to its good biocompatibility, low toxicity and biodegradability.[11,12] The degradation products of chitosan are nontoxic, nonimmunogenic, and noncarcinogenic. Chitosan microspheres cross-linked with glutaraldehyde were shown to be long-acting biodegradable carriers suitable for use in microspheres delivery system.[13—16]

The aim of the present work here was to design a new colonic drug delivery system which combines two approaches: pH-sensitive delivery and biodegradation by bacterial enzymes in the colon environment. Coating made the membrane coating began dissolve gradually above pH 6.8, and the chitosan MS were exposed. Then the exposed microspheres were biodegraded by bacterial enzymes in the colon. In this experiment, 5-Fu loaded pH-enzyme Di-dependent chitosan MS were successfully prepared by an emulsion-chemical cross-linking and coating technique. 5-Fu loaded chitosan MS was optimized by an orthogonal design. The drug release behaviors *in vitro* and pharmacokinetics *in vivo* of drug loaded pH-enzyme Di-dependent chitosan MS were further evaluated and their correlations were discussed in detail.

## Experimental

**Materials** Chitosan (Mw: 100000) with 92.3% deacetylation degree was a present from Ocean University of China. 5-Fu was obtained from Shenyang Jiqi Pharmaceutical Co. Ltd. TEFOSE-63 (ethylene glycol and polyoxyethylene glycol palmitostearate) and Eudragit S100 (methacrylic acid copolymer B) were kindly donated by Gattefosse (France) and the Rohm (Germany), respectively. Glutaraldehyde was purchased as a 25% aqueous solution from Sigma Chemical Co. (U.S.A.). Acetic acid was obtained from Tianjin Concord Tech. Co., China and other reagents were all of analytical grade.

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**Preparation of 5-Fu Loaded pH-Enzyme Di-Dependent Chitosan MS** The 5-Fu loaded pH-enzyme Di-dependent chitosan MS was prepared by two steps, the preparation of chitosan MS and the coating of chitosan MS. Microspheres were prepared by emulsion-chemical cross-linking technique, modified from previously methods.[17] 5-Fu loaded chitosan MS were prepared with drug : polymer ratios (w/w) of 1 : 10, 1 : 5 and 1 : 2 (Table 1). Briefly, chitosan was dissolved in 1% (v/v) acetic acid solution to obtain a polymer solution at a concentration of 2% (w/v), respectively. Different amount of 5-Fu was dissolved in the polymer solution to form a drug/polymer solution (aqueous phase). This solution was added dropwise into 30 ml liquid paraffin (oil phase) containing 2% (w/w) TEFOSE-63. The mixture was stirred at 300 rpm with a mechanical stirrer for 30 min to form W/O emulsion. Later, 10 or 20 ml of 25% glutaraldehyde solution was added drop-wise slowly into the medium and then further coss-linked for 2 h. The microspheres formed were collected by pressure reduction filtration, and washed with isopropanol, sodium bisulfite and petroleum ether twice separately and then dried in a vacuum desiccator for 12 h.

5-Fu chitosan microspheres (100 mg) were then coated with Eudragit S100 in an aqueous phase using a fluid-bed spray coater to given 20% weight gain. Coating was carried out at an air pressure of 0.2 MPa at a spray rate of 0.25 ml/min. The inlet and outlet temperatures were 30 and 25 °C, respectively. Eudragit S100 suspension was prepared as recommended by the supplier. This solution was transported to the nozzle using a peristaltic pump. The coated beads were stored in a sealed polythene bag before use.

**Drug Content of Microspheres** The 5-Fu content of chitosan MS was determined as follows: First, 50 mg chitosan MS were dispersed in 5 ml of 0.1 M NaOH, and were dissolved by mashing with a glass mortar using a pestle. The mixtures were placed into a 10 ml tube with a cap and broken up by ultrasonication for 30 min (400 W) and shaked for 24 h at room temperature. After centrifugation (4000 $rpm^{-1}$ for 15 min), 1 ml of supernatant was transferred into a 25 ml flask. After neutralized with 0.1 M HCl, the solution was diluted to 25 ml with methanol and determined using the HPLC method.

The chromatographic system consisted of a pump (Shimadzu LC-10AD), a UV detector (Shimadzu SPD-10A), a 20 $\mu$l loop (Rhenodyne model 7725i). A Diamonsil™ C18 column (200 mm×4.6 mm, 5 $\mu$m, Dikma Technologies) and a Phenomenex C18 securityguard (4 mm×3.0 mm, 5 $\mu$m, Torrance) were utilized for drug separation, while using methanol–1% acetic acid (1 : 100,v/v) as mobile phase for determination of 5-Fu. The flow rate and UV wavelength were 0.8ml/min and 266nm, respectively.

The efficiency of drug entrapment (EE) and drug load content (LD) of MS were calculated by Eqs. 1 and 2,

$$\text{drug loading (\%)} = \frac{\text{weight of drug in MS}}{\text{weight of MS}} \times 100\% \quad (1)$$

$$\text{entrapment efficiency (\%)} = \frac{\text{drug loading}}{\text{theoretical loading}} \times 100\% \quad (2)$$

**Characterization of the Chitosan MS** The morphology of the MS before and after the coating was characterized by scanning electron microscopy (Jeol 5200 SEM). Mean diameters (MD) were measured using a Nicomp 380-Submicron Particle Sizer (Particle Sizing Systems, Santa Barbara, CA, U.S.A.) at a fixed angle of 90° at 25 °C.

The MSs were sealed for storage at 25 °C for 6 months. The drug content, average size and entrapment efficiency were determined.

***In Vitro* Release Study** The 5-Fu loaded MS and pH-enzyme Di-dependent chitosan MS were tested for drug release for 2 h in 0.1 M HCl (250 ml), as the average gastric emptying time is about 2 h,[18] respectively. Then the dissolution medium was replaced with pH 6.8 phosphate buffer (250 ml) and tested for drug release for 3 h, as the average small intestinal transit time is about 3 h. Next, pH 7.4 phosphate buffers (250 ml) was used to test for drug release for 19 h. Six dialysis bags containing 2±0.1 ml saline were introduced into the system. At desired times, samples (0.5, 1, 2, 3, 5, 8, 10, 12, 24 h) were withdrawn, filtered and assayed for drug released as described above.

***In Vivo* Study** Wistar rats (male and female, 12 weeks old, 200±30 g) were provided by the Animal Center of Shenyang Pharmaceutical University (the experimental protocol was proved by the Ethics Review Committee for Animal Experimentation of Shenyang Pharmaceutical University). Before administration, the rats were fasted overnight but were allowed free access of water and *libitum*. The 5-Fu coated microspheres or 5-Fu aqueous solution was administrated orally to rats (20 mg/kg). At appropriate intervals, blood samples (approximately 0.4 ml) were drawn by puncture of the retroorbital sinus. Blood samples were collected in heparinized tubes and stored at −20 °C as soon as possible until assay. Specimens were thawed and allowed to reach room temperature before analysis.

The area under the drug concentration–time curve from 0 to 24 h ($AUC_{0—24}$) was calculated using the trapezoidal rule. The maximal plasma concentration of drug ($C_{max}$) and the time to reach maximum plasma concentration ($T_{max}$) were directly obtained form plasma data. The data from different formulations were compared for statistical significance by one-way analysis of variance (ANOVA). All results were expressed as mean±S.D.

## Results and Discussions

**Preparation of 5-Fu Loaded Chitosan Microspheres** As shown in Table 1, the results demonstrated that drug/chitosan ratios and the amount of crosslinking agent affected the microshperes characteristics. As the ratio of drug/chitosan decreased from 1 : 2 to 1 : 10, encapsulation efficiency decreased; this is due to the fact that higher amount of polymer would produce small size droplets with increased surface area, such that diffusion of drug from such microspheres will be fast, resulting in the loss of drug with a consequent lowering in encapsulation efficiency. A similar finding was reported before by Raghavendra C.[19] As for the effect of extent of crosslinking on the entrapment efficiency data of the microspheres, with increasing amount of crosslinking agent, encapsulation efficiency decreased. For instance, for microspheres crosslinked with 10 and 20 ml of glutaraldehyde (formula 1 to 3 and formula 4 to 6), entrapment efficiencies are, respectively, 40.57, 33.96% and 35.58, 29.53%. It maybe results of higher crosslinking amount, since microspheres are more rigid and the free volume space within the matrix would decrease, resulting in reduced encapsulation efficiency.

According to the previous study, under the same amount of glutaraldehyde (10 ml), the cross-linking degree increased with increasing cross-linking time (data were not shown). The cross-linking degree significantly increased from 2 to 4 h cross-linking time ($p<0.05$), but not from 4 to 8 h. It is hypothesized that this is due to the outer layers of microspheres

Table 1. Results of Encapsulation Efficiency (EE) and Mean Diameter (MD) of Chitosan Microspheres

| Formula | Chitosan conc. (%) | Drug/chitosan ratio | Crosslinking agent amount (ml) | EE (%) | MD ($\mu$m) |
|---|---|---|---|---|---|
| 1 | 2 | 1 : 2 | 10 | 40.57±0.92 | 218.6±3.21 |
| 2 | 2 | 1 : 5 | 10 | 36.06±0.82 | 197.9±1.56 |
| 3 | 2 | 1 : 10 | 10 | 33.96±0.91 | 173.2±2.07 |
| 4 | 2 | 1 : 2 | 20 | 35.58±1.02 | 199.4±1.96 |
| 5 | 2 | 1 : 5 | 20 | 32.72±0.87 | 187.9±1.87 |
| 6 | 2 | 1 : 10 | 20 | 29.53±0.84 | 160.1±1.90 |

being cross-linked, thereby limiting cross-linking of inner layers. Since the degree of crosslinking changed significantly from 2 to 4 h at 10 ml glutaraldehyde, 4 h cross-linking time was selected in order to observe the effect of glutaraldehyde concentration on the formulation.

Particle size analysis of 5-Fu microspheres showed that the mean microsphere diameter was also affected by drug/polymer ratio and amount of crosslinking agent in all the formulations. When the polymer ratio increased (drug/polymer ratio 1/10, 1/5, 1/2), the size of microspheres increased due to increase amount of the drug in internal phase. As the drug amount was increased and the polymer ratio decreased, a more viscous internal phase occurred. During the emulsification process, the internal phase was hardly dispersed in the outer phase and larger microspheres were produced. When the amount of drug was decreased as the polymer ratio increased (drug/polymer ratio 1/2, 1/3, 1/4), the size of microspheres decreased due to reduced viscosity of the internal phase. These findings are similar to those reported previously.[20] Amount of crosslinking had an effect on particle size. For instance, for microspheres containing 1/5 drug/polymer ratio (formula 2, 5), with increasing crosslinking by glutaraldehyde *i.e.*, 10 and 20 ml glutaraldehyde, particle size decreased from 197.9 to 187.9 $\mu$m and similar trend is observed for formulations. This is attributed to the fact that with an increase in the amount of glutaraldehyde, shrinkage of particles might have occurred leading to the formation of smaller particles.[21]

As the polymer concentration was increased from 2 to 3% (data were not shown), similar trends were found as above formulations. During the experiment, we found that the viscosity of the chitosan solution was one of the important factors related to microsphere formulations. MSs did not form at low concentration of chitosan solution. A highly concentrated solution made the dropping process difficult and MS could not readily be formed. On the other hand, during the emulsification process, the internal phase was hardly dispersed in the outer phase and larger microspheres were produced.

During the preparation, we found that MS could not be obtained due to aggregation when using liquid paraffin with no TEFOSE-63 as an oil phase. An addition of TEFOSE-63 can decrease the viscosity of oil phase and can stabilize the W/O emulsion. Also, process variables such as stirring rate affected 5-Fu chitosan microsphere formulations.

In order to get the optimized formulation to prepare 5-Fu loaded chitosan MS, the concentration of chitosan, drug/chitosan ratio, amount of crosslinking agent, and dispersion speed during emulsification were chosen as the most influential factors (labeled as A, B, C, D in Table 2). Taking the EE as an index, the four factors were investigated at three different levels. The L9($3^4$) orthogonal design was established as shown in Tables 2 and 3. The range, describing the relationship between index and each factor, was drawn to select the optimum ingredient composition which reflected the degree that various factors affected the index. The ranking of the four factors in this experiment was C>B>A>D, and the individual levels within each factor were ranked as: A: 2>1>3; B: 1>2>3; C: 2>1>3; D: 2>1>3. The optimized formulation should be $A_2B_1C_2D_2$ according to the analytical results using Orthogonality Experiment Assistant Version 3.1 (Sharetop Software Studio).

Table 2. The Levels of Experimental Factors

| Factors | A | B | C | D |
|---|---|---|---|---|
| Levels | Conc. of CS (%) | 5-Fu : CS (w/w) | Crosslinking agent amount (ml) | Dispersion rate (r/min) |
| 1 | 2 | 1 : 8 | 10 | 200 |
| 2 | 2.5 | 1 : 5 | 15 | 300 |
| 3 | 3 | 1 : 4 | 20 | 400 |

Table 3. Orthogonal Experiment Design and Entrapment Efficiency Result

| | A | B | C | D | EE (%) |
|---|---|---|---|---|---|
| 1 | 1 | 1 | 1 | 1 | 34.51 |
| 2 | 1 | 2 | 2 | 2 | 35.02 |
| 3 | 1 | 3 | 3 | 3 | 10.29 |
| 4 | 2 | 1 | 2 | 3 | 42.55 |
| 5 | 2 | 2 | 3 | 1 | 17.67 |
| 6 | 2 | 3 | 1 | 2 | 26.64 |
| 7 | 3 | 1 | 3 | 2 | 22.48 |
| 8 | 3 | 2 | 1 | 3 | 23.50 |
| 9 | 3 | 3 | 2 | 1 | 28.68 |
| $\bar{I}$ | 26.61 | 33.18 | 28.22 | 26.95 | |
| $\bar{II}$ | 28.95 | 25.40 | 35.42 | 28.05 | |
| $\bar{III}$ | 24.89 | 21.87 | 16.81 | 25.45 | |
| R | 4.066 | 11.31 | 18.61 | 2.6 | |

A: concentraiton of chitosan (w%); B: drug : chitosan (w/w); C: crosslinking agent amount (ml); D: dispersion speed (r/min); $\bar{I}$: average of entrapment efficiency value of level 1 of factors; $\bar{II}$: average of entrapment efficiency value of level 2 of factors; $\bar{III}$: average of entrapment efficiency value of level 3 of factors; R: range of the maximum and minimum.

**Characteristics of 5-Fu Loaded pH-Enzyme Di-Dependent Chitosan** The curve of particle size distribution of optimized 5-Fu loaded microspheres before and after coating is given in Fig. 1. The mean particle diameters of chitosan MS before and after coating were 196.7 $\mu$m and 278.4 $\mu$m, respectively. Figures 2a and b show the scanning electron micrography photos of microspheres before and after coating. Figure 2c illustrates the appearance of a MS before coating. Microspheres were spherical and have porous surface and roughness. Free drug crystals were not seen on the surface of microspheres. The presence of pores in MS surface was mentioned earlier for the use of other types of polymers.[22] In Fig. 2d, micrographs show the smooth surface of MS after they are coated.

Table 4 gives the data of content, EE, and particle sizes of 5-Fu loaded pH-enzyme Di-dependent chitosan MS after 0, 1, 3 and 6 months of storage at room temperature. The 5-Fu MS showed sufficient long-term stability with no significant changes of mean diameter or drug leakage ($p>0.05$) after storage at room temperature for 6 months. There was also no visible aggregation in system during storage.

**Drug Release Behavior** Figure 3 shows the release of 5-Fu from uncoated MS and optimum coated MS over 24 h at pH 1.2 followed by a 5-Fu triggered release at pH 6.8 and pH 7.4, respectively. Drug release from MS before and after coating indicated that there was significant difference in the release rates before and after coating. About 50% of drug in uncoated MS have released within 4 h, suggesting the burst release effect in uncoated MS, which indicated the drug in uncoated MS has released before they reached colon. As for

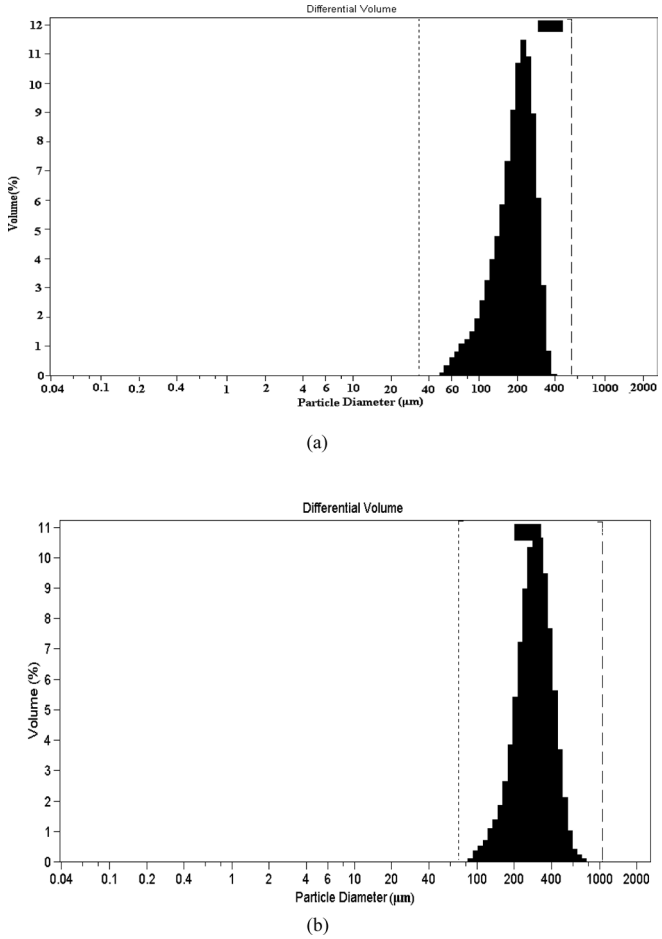


Fig. 1. Particle Size Distribution of the Optimized Chitosan Microspheres before (a) and after (b) Coating

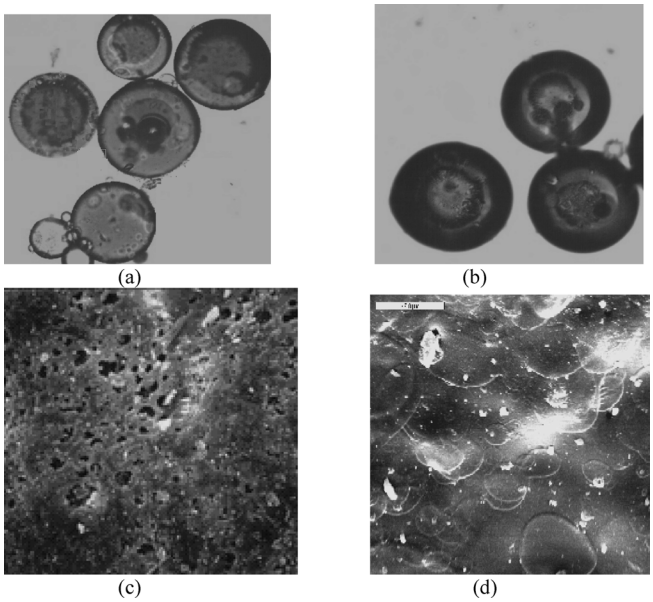


Fig. 2. Scanning Electron Microphotographs of: (a) Dried Chitosan MS (×40); (b) Coated Chitosan MS (×40); (c) Dried Chitosan MS (×1500); (d) Coated Chitosan MS (×1500)

the coated MS, a slower drug release is achieved. The initial release of drug from the coated MS was low. Little 5-Fu could be measured in the pH 1.2 medium for 2 h, illustrating

Table 4. The Storage Stability of 5-Fu Coated MS (20±5 °C, $n$=5)

| Storage conditions (°C) | Time (month) | Content (%) | EE (%) | Mean diameter ($\mu$m) |
|---|---|---|---|---|
| 20±5 | 0 | 3.84 | 42.15±0.3 | 278.4±14.8 |
| | 1 | 3.83 | 42.08±0.2 | 279.1±12.8 |
| | 3 | 3.81 | 42.10±0.4 | 279.4±9.6 |
| | 6 | 3.81 | 41.26±0.4 | 279.9±10.3 |

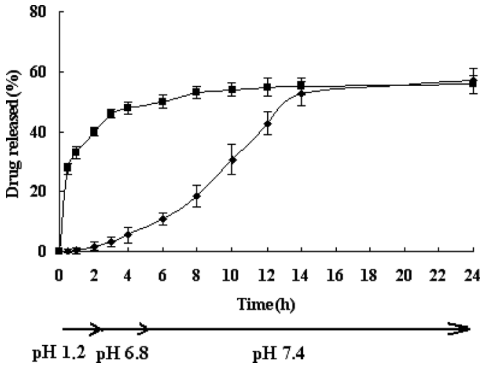


Fig. 3. The Release Profiles of 5-Fu from Chitosan MS Coated with Eudragit S 100 and Uncoated MS in the Simulated Gastrointestinal pH Conditions ($n$=6)

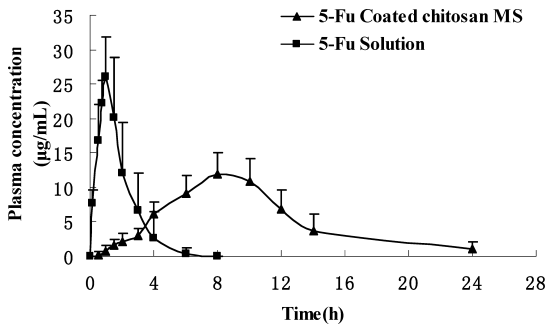


Fig. 4. The Mean Concentration–Time Curve after a Single Oral Administration of 5-Fu Solution and 5-Fu Coated Chitosan MS

Data are means±S.D., $n$=5.

the coated MS suppressed 'burst effect' to some extent. Only 1.78±0.58% was released after 2 h. After 24 h about 56.89% had been released. Enteric coating using Eudragit S-100 (soluble above pH 7.0, according to the supplier specifications) has traditionally been used to prevent drug release in the upper gastrointestinal tract.[23] The results of present study prove that the 5-Fu was protected from acid in gastric juice by a membrane coating. Above pH 6.8, the membrane coating began to dissolve gradually, and the chitosan MS were exposed. Then the drug release from MS followed diffusion and erosion mechanism. In our *in vitro* drug release study, there were no enzymes used in the dissolution medium, so the cumulative drug release was less 60% in 24 h. Nevertheless, based on the results of the *in vitro* drug release studies, we can conclude that the coated chitosan MS may provide targeting of 5-Fu in the colon.

**Pharmacokinetic Evaluation** Figure 4 shows the plasma concentration *vs*. time profiles of 5-Fu after administration of coated-MS and 5-Fu aqueous solution to rats at a dose of 20 mg/kg (calculated by 5-Fu), respectively. Colon-specific absorption of released 5-Fu affected its pharmacoki-

netic parameters. After oral administration of the 5-Fu solution, the drug was detected rapidly in plasma. The maximum concentration of 5-Fu was 30 $\mu$g/ml after about 1 h. Thereafter, the plasma concentration decreased quickly and the drug was not detectable as soon as 8 h. The elimination half-life was 0.74±0.14 h. This was maybe due to the precipitation of drug in the stomach and consequently decreases the overall amount of 5-Fu absorbed. Similar results have previously been reported by Zinutti *et al*.[24] In the case of pH-enzyme Di-dependent chitosan MS, the 5-Fu level reached within about 8 h and then gradually decreased for the followed 16 h, which indicated that with the equivalent dosage, the 5-Fu MS exhibited an obvious prolonged acting time. The longer residence time of released drug in colon resulted in sustained absorption.[25,26]

The non-compartmental pharmacokinetic parameters of coated 5-Fu loaded chitosan MS and 5-Fu solution are summered in Table 5. The $C_{max}$, $T_{max}$, $T_{1/2}$ and $AUC_{0—\infty}$ were significantly different from those of the aqueous solution. The steady low plasma drug concentrations of pH-enzyme Di-dependent chitosan MS may provide not only a safety benefit by reducing the magnitude of peak plasma drug levels,[27] but may also result in sustained drug exposure of tumor.[28] The high relative bioavailability (238.49±65.36%) in MS was probably a consequence of the protection of enteric polymer coatings and the slow degradation of chitosan in colon by the action of bacterial enzymes.

Figure 5 compares the *in vitro* and *in vivo* 5-Fu release profiles, the former was plotted as cumulative area under plasma 5-Fu curve normalized as percent of the total area between hours 0 to ∞ h (total area under the curve was 130.60 $\mu g \cdot ml^{-1} \cdot h$). The overall *in vivo* estimated rate was faster than the *in vitro* release rate after 8 h. We attributed the more bacterial enzymes in colon, small weight of the drug, as well as the obvious prolonged acting time during *in vivo* release. However, the *in vitro* release rate of 5-Fu correlated rather well with the estimated *in vivo* release ($r$=0.9903, $n$=5, $p$<0.001), as shown in Fig 5, small panel. The established *in vivo–in vitro* correlation may be utilized to predict the pharmacokinetic response of 5-Fu loaded pH-enzyme Di-dependent chitosan MS once the *in vitro* concentration have been determined.

Table 5. 5-Fu Pharmacokinetic Parameters after Oral Administration (Mean±S.D., $n$=5)

| Parameter | Solution | Coated-MS |
|---|---|---|
| $C_{max}$ ($\mu g \cdot ml^{-1}$) | 30.16±2.87 | 13.23±2.82* |
| $T_{max}$ (h) | 1.04±0.42 | 8.33±1.51* |
| $T_{1/2}$ (h) | 0.74±0.14 | 4.43±1.99* |
| $AUC_{0—\infty}$ ($\mu g \cdot h \cdot ml^{-1}$) | 54.76±20.91 | 130.60±34.42** |
| Relative bioavailability (%) | 100 | 238.49±65.36%** |

* $p$<0.05 *vs*. solution group. ** $p$<0.01 *vs*. solution group.

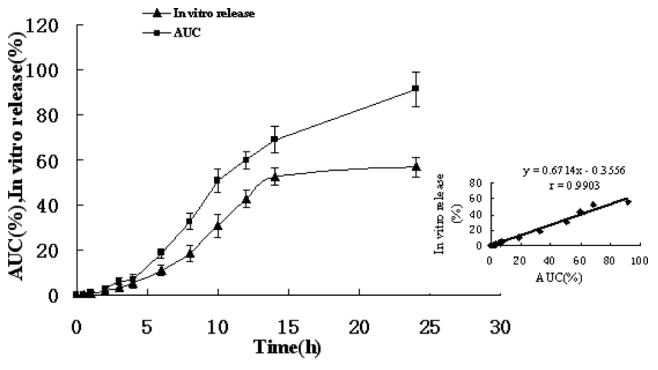


Fig. 5. *In Vitro* Release of 5-Fu Loaded Microspheres ($n$=3) under Mimicking Stomach to Colon Transit Conditions and Comparison with *in Vivo* Release Profile ($n$=6) (Plotted as Cumulative Area under Plasma 5-Fu Curve Normalized as Percent of the Total Area under the Curve); *in Vitro–in Vivo* Correlation Plot (Small Panel)

## Conclusion

In this study, a novel 5-Fu loaded pH-enzyme Di-dependent chitosan MS were prepared by an emulsion-chemical cross-linking and coating technique, respectively. The optimum formulation was prepared by orthogonal design. Drug release studies *in vitro* demonstrated that the coated chitosan MS may provide targeting of 5-Fu in the colon. An oral pharmacokinetic study was conducted in rats and the results showed that MS produced a significant improvement in the bioavailability of 5-Fu compared with 5-Fu solution. The *in vitro* release rate of 5-Fu correlated rather well with the estimated *in vivo* release. It appears that pH-enzyme Di-dependent chitosan MS offer a promising delivery system for the enhancement of the bioavailability of 5-Fu.

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