Journal of Applied Polymer Science

Microencapsulation of Nanoparticles with Enhanced Drug Loading for pH-Sensitive Oral Drug Delivery for the Treatment of Colon Cancer

Yichao Wang,[1] Puwang Li,[1,2] Zheng Peng,[1,2] Feng Hua She,[1] Ling Xue Kong[1]

[1]Institute for Frontier Materials, Deakin University, Waurn Ponds, Vic 3216, Australia

[2]Agricultural Product Processing Research Institute, Chinese Academy of Tropical Agricultural Sciences, Zhanjiang 524001, People's Republic of China

Correspondence to: L. X. Kong (E-mail: lingxue.kong@deakin.edu.au)

ABSTRACT: Drug-delivery carriers must endure harsh pH conditions in the gastrointestinal tract and still maintain a high drug loading (DL) for oral therapeutic drugs to be effectively delivered to the colon area. In this research, a pH-sensitive drug-delivery system with an enhanced DL was developed by the coating of 5-fluorouracil (5-FU)-loaded poly(lactide-*co*-glycolide) (PLGA) nanoparticles (NPs) with Eudragit S100 with an oil-in-oil solvent evaporation technique. The enhanced DL and encapsulation efficiency were achieved by the optimization of the fabrication parameters and by the use of particles of a proper size. A DL of 5.8% was obtained by a moderate initial drug feeding, a high volume ratio of the outer water phase to the organic phase, and by the adjustment of the pH value of the outer aqueous phase to the isoelectric point of 5-FU. An *in vitro* drug-dissolution test showed that the coating of the Eudragit S100 microspheres could effectively prevent drugs from being released in an environment with a pH lower than 7. The PLGA NPs showed an initial burst release followed by a slow and sustained release over an extended period of over 120 h at pH value of 7.4. Therefore, the prepared systems have great potential for practical applications in the treatment of colorectal cancer. © 2012 Wiley Periodicals, Inc. J. Appl. Polym. Sci. 129: 714–720, 2013

KEYWORDS: biodegradable; biocompatibility; drug-delivery systems

Received 12 May 2012; accepted 10 September 2012; published online 26 November 2012
DOI: 10.1002/app.38582

INTRODUCTION

Well-designed oral drug-delivery vehicles using biodegradable and biocompatible polymers for the treatment of colorectal cancer have attracted increasing attention, as they provide a sustained and controlled drug release and reduce side effects.[1] Furthermore, oral colonic drug-delivery is convenient to administer and painless to patients compared to traditional treatments, such as surgery and radiation therapy.[2] Among these oral delivery systems, the formulation of nanoparticles (NPs) or microspheres (MSs) has been one of the most promising technologies. However, for NP carriers, a large amount of drug can be lost in the upper gastrointestinal tract (GIT), whereas less functionality can be achieved for microspherical vehicles.[3] Therefore, the design of a new drug-delivery vehicle that incorporates the formulation of NPs and MSs will prevent drug loss in the delivery route and improve functionality.

Drug-delivery vehicles with a high drug loading (DL), particularly in the form of NPs,[4,5] are practical for the administration of therapeutic drugs for the treatment of colorectal cancer. The low DL of NPs has always been a concern,[6] although they provide sustained drug release and can penetrate cancerous cells much more easily.[7] NP formulations are normally introduced into human body via the route of injection or a suppository, as encapsulated drugs can easily be released in the upper GIT when they are administered orally.[8] Therefore, for effective oral administration of nanoencapsulated therapeutic cancer drugs to the colon, the NPs must have a high DL for sustained release and for them be delivered to cancerous cells with minimum loss.

The major challenge of delivering NPs to colorectal cancer cells is the encapsulation of sufficient therapeutic drug into NPs with particle sizes between 70 and 200 nm as particles in this specific size range have been regarded as ideal for cancer treatment,[9] especially when they encapsulate hydrophilic drugs, such as the widely used 5-fluorouracil (5-FU), into hydrophobic materials. In fact, the maintenance of a high DL is important in the practical application as the number of drug administrations for patients can be effectively minimized.[10] The intrinsic difference in the chemical properties causes a lower affinity to each other. When hydrophilic therapeutic agents are encapsulated into

hydrophobic materials, there is a great repulsion between the two phases. It is challenging for hydrophilic drug to be encapsulated into hydrophobic material to form NPs.[11,12] Therefore, a variety of methods for improving the DL for drug-delivery carriers have been developed by the modification of therapeutic drugs and encapsulation materials. McCarron and Hall[12] substituted the hydrophilic drug 5-FU with its prodrug 1-alkylcarbonyloxymethyl in the fabrication process; this resulted in a dramatic increase in DL from 3.68 to 47.23%. Zhang and Feng[13] conjugated the hydrophilic groups of tocopheryl poly(ethylene glycol) succinate (TPGS) onto the hydrophobic encapsulation material polylactide/poly(lactide-*co*-glycolide) (PLGA) and synthesized a copolymer that enhanced the hydrophilicity of the drug carrier and the affinity to the hydrophilic encapsulated drugs. However, few results have been reported on the effects of the fabrication parameters on the DL, encapsulation efficiency (EE), NP size, and their correlation on the basis of the same conditions. The double-emulsion and solvent evaporation method ($W_1/O/W_2$, where W_1 and W_2 are the aqueous solutions and O is the organic phase), a popular and widely used method in pharmaceutical research and production, has demonstrated a high DL.[14] The determination of the formulation procedures needed to obtain an optimized combination of DL and particle size is essential for the production of effective nanodrug deliver carriers.

Another challenge for effective drug delivery for the treatment of colorectal cancer is the delivery of enough therapeutic drug encapsulated in NPs to the cancerous cells in the colonic area. The delivery route is a harsh and complicated environment, and encapsulated drugs are easily lost in the varying pH environments of the GIT.[15] Some polymeric MSs have demonstrated a strong pH dependence; this means that they only take effect in environments of a certain pH value. Eudragit S100, an anionic polymer synthesized from methacrylic acid and methacrylic acid methyl ester, is a commonly used polymer for the fabrication of functionalized MSs.[11] It is insoluble in acids and pure water and only dissolves in aqueous solution at pH 7 or higher;[16,17] this is ideal for colonic drug delivery. The microencapsulation of the NPs with pH-sensitive Eudragit S100 should fully protect the encapsulated drugs from being released in the upper gastrointestinal delivery route.

Therefore, PLGA NPs with enhanced therapeutic 5-FU DL and a pH-sensitive coating were fabricated in this study. DL, EE, and the particle size were all tested. Optimized fabrication parameters were determined to prepare enhanced drug-loaded NPs with a suitable particle size. The cumulative drug release in different pH environments was also recorded to demonstrate the effectiveness of the system.

EXPERIMENTAL

Materials

PLGA–COOH 50/50, with an average molecular weight of 15,000, was purchased from Ji'nan Daigang Biological Co., Ltd. (Shandong, China). 5-FU, methylene chloride (DCM), and poly(vinyl alcohol) (PVA), with a 86.7–88.7% hydrolysis degree and a molecular mass of 31,000, were ordered from Sigma Chemical Co. (Castle Hill, NSW, Australia) Eudragit S100 was obtained from You Pu Hui Co., Ltd. (Shenzhen, China). The

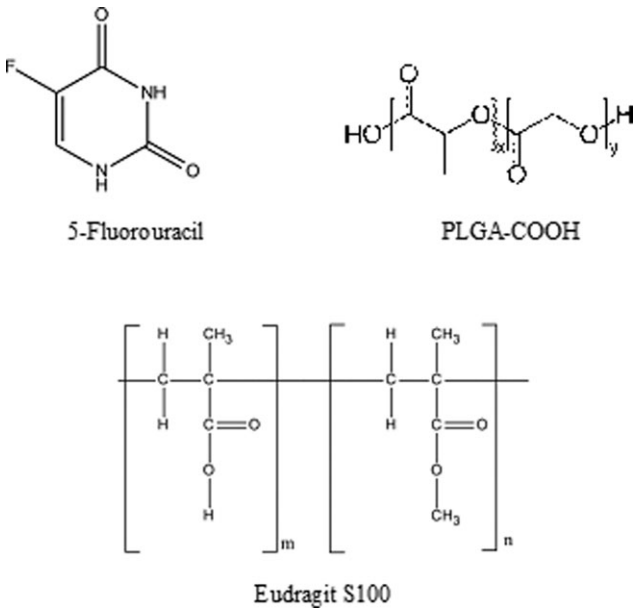

Figure 1. Chemical structures of 5-FU, PLGA, and Eudragit S100.

structures of the main chemicals are described in Figure 1. All of the chemicals were analytical grade and were used without further purification.

Preparation of the PLGA NPs and Eudragit S100 MSs

The PLGA NPs loaded with 5-FU were prepared with a modified $W_1/O/W_2$ multiple emulsion and solvent evaporation technique (Figure 2).[18] Briefly, 100 mg of PLGA was dissolved in 6 mL of DCM. 5-FU was dissolved into a water solution to obtain the inner aqueous phase. Into the organic phase (O), the aqueous drug solution (W_1) was emulsified with a probe sonicator (Shengxi Instrument Co., Shanghai, China) for 2 min with a 30% amplitude to form a W_1/O emulsion. The first emulsion was incorporated into an aqueous phase containing PVA (external phase, W_2) and sonicated for 1 min. The resulting $W_1/O/W_2$ emulsion was stirred with a magnetic stirrer for 5 h to allow solvent evaporation and particle hardening. The NPs were then separated by ultracentrifugation at 12,000 rpm for 20 min and washed with distilled water three times to remove 5-FU on the surface of the particles and excessive surfactant; this was followed by further centrifugation to eliminate the washing solution. Finally, the NPs were collected from a freeze dryer and were preserved in a desiccator for evaluation and analysis.

The core PLGA NPs were then coated with the pH-sensitive polymer, Eudragit S100. The fabrication process was an oil-in-oil solvent evaporation technique. A mixture of methanol and acetone was used as the organic phase as it dissolved the Eudragit S100 properly and, at the same time, kept the PLGA NPs intact.[19] The fabrication process was as follows: core PLGA NPs were dispersed in the Eudragit S100 solution (10% w/v). The dispersion of PLGA NPs into Eudragit S100 was carried out by sonication with a probe sonicator. Then the formed dispersions were emulsified in liquid paraffin containing 1% v/v Span 80 by a mechanical stirrer. Such agitation was continued for 5 h to ensure that all of the solvents were evaporated. The

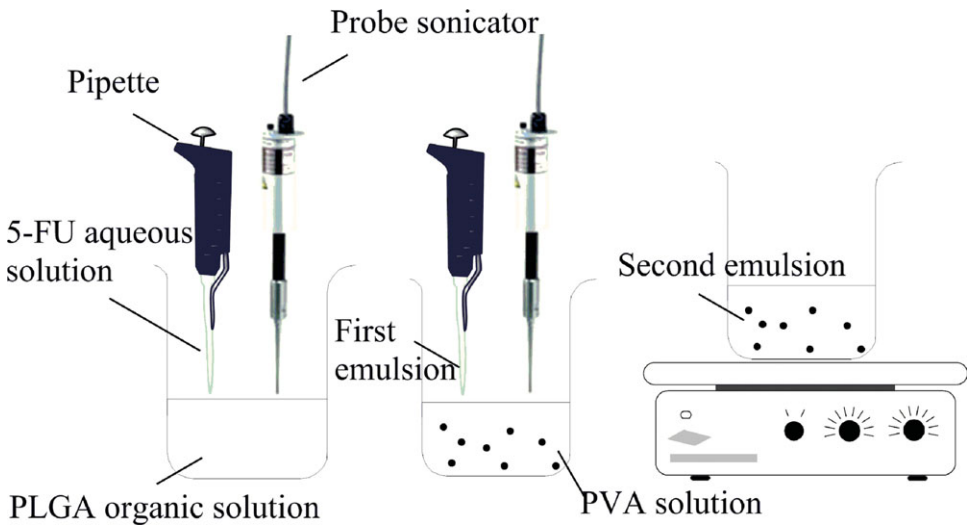

Figure 2. Fabrication process of the 5-FU-loaded PLGA NPs. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

encapsulated MSs were obtained by filtration and were washed three times with petroleum ether to remove residual liquid paraffin. The samples were finally dried *in vacuo* for 24 h.

Characterization of the NPs

To determine the 5-FU drug entrapped in PLGA NPs, an indirect method was carried out by measurement of the drugs that were not encapsulated. The prepared NP solution was centrifuged at 12,000 rpm for 20 min. Then, the supernatant was collected and tested with an ultraviolet–visible spectrometer at a wavelength of 265 nm. DL and EE were calculated with the following equations:[20]

$$\mathrm{DL}(\%) = \frac{A - B}{C} \times 100 \quad (1)$$

$$\mathrm{EE}(\%) = \frac{A - B}{A} \times 100 \quad (2)$$

where A is total amount of feeding drug, B is the amount of drug in the supernatant solution after centrifugation, and C is the weight of the prepared NPs. During this test, all measurements were made in triplicate, and the mean values are shown in the results.

The shape and surface morphology of the NPs and MSs were characterized with a Zeiss Supra 55 VP FEG scanning electron microscope (Zeiss SMT, Oberkochen, Germany). The particle size was determined by a dynamic light scattering (DLS) spectrometer with a Zetasizer Nano ZS (Malvern Instruments, Worcestershire, United Kingdom). The spectrometer was equipped with a He–Ne laser, and the digital correlator inside had a wavelength of 633 nm. All of the measurements were carried out at room temperature.

In Vitro Drug Release from the PLGA NPs and Eudragit S100 MSs

The main parts that drug carriers pass through in the human GIT are the stomach, the duodenum and the small intestine, the colon, and the rectum, whose pH conditions are 1.2, 4.5, 6.8, and 7.4, respectively. The transition times to pass through each part of the GIT are 1, 2, and 2 h and the time until drug carriers are expelled from human body.[16]

Therefore, the release of 5-FU from the PLGA NPs and Eudragit S100 MSs was performed in a pH progression medium simulating the conditions of different parts of a real GIT. In the release study, two buffer solutions, HCl buffer and phosphate-buffered saline (PBS), were selected. The pH of the medium increased gradually from pH 1.2 (HCl buffer) for the 1st h of drug release, pH 4.5 (PBS) for the next 2 h, pH 6.8 (PBS) for the following 2 h, and pH 7.4 (PBS) until the end of the test.

There were a number of distinct steps in the release test procedure. First, the as-prepared MSs or NPs were placed in a dialysis bag whose molecular weight cutoff was 8000 Da. The tests were performed in a constant-temperature shower mixer at 100 rpm at 37°C with 50 mL of dissolution solution, and the release was measured in corresponding buffer solutions at different pH values. After a fixed time interval, 3.5 mL of the solution was taken out and diluted to a 30-fold volume. Then, the ultraviolet absorbance at 265 nm was tested. After that, 3.5 mL of fresh buffer solution was added to the release medium to maintain a constant solution volume. After n samples were taken, the drug that was released could be calculated as $C = C_n \times 50 + (C_1 + C_2 + \ldots + C_{n-1}) \times 3.5$, where C_i is the concentration of the solution of the nth sample.

Calculations and Statistics

The results were recorded as the mean plus or minus the standard deviation (SD). The data were collected from three different batches of samples prepared with certain fabrication parameters. An analysis of variance one-way analysis (Origin 8.0 software, Northampton, USA) was used to conduct an analysis of significance between the mean values. Probability values of $p < 0.05$ were considered to be significant.

RESULTS AND DISCUSSION

Effect of the Nanofabrication Parameters

Theoretical Drug Loading (TDL) of 5-FU. The practical drug loading in the NPs depended on the TDL and the synthesis conditions. Four TDLs of 5, 10, 15, and 20% w/w were selected to examine the TDL effects on the practical DL and EE of the NPs.

An increase in TDL had a notable effect on the actual DL, EE, and particle size (Table I). As the TDL increased from 5 to 20%, the particle size increased gradually from 189.2 to 233.6 nm, respectively, because of the increase in the actual drug content in the NPs.[10] The particles fabricated with the double-emulsion and solvent evaporation method were on the nanoscale and had a relatively low polydispersity index (PDI < 0.19); this indicated a narrow particle size distribution.

When the TDL was low, it contributed significantly to the actual DL and EE. The DL ranged from 2.4 to 6.8% and increased almost proportionally when TDL was lower than 15% (Table I). However, a further increase from 15 to 20% in TDL resulted in only a very small increase in DL; this indicated that there was a saturated DL for the PLGA and 5-FU drug-delivery carrier.

However, as observed from Table I, an improvement in DL heavily compromised EE, particularly when TDL was higher than 15%, in which only less than 19.2% of 5-FU was encapsulated in the NPs. The stability of the first emulsion was reduced when the initial drug feeding was increased;[21] this resulted in more drug loss in the fabrication process and a high manufacturing cost. So a compromise needed to be obtained to get a relatively high DL and EE. In this experiment, we found an optimal formulation with a TDL of 10%, where a balance of relatively high DL and EE was reached.

Volume Ratio of the Outer Water Phase to the Organic Phase. In the $W_1/O/W_2$ fabrication process, the organic phase is the dispersing phase, whereas the outer water phase is the continuous phase that is used to harden the NPs and promote the organic solvent to be evaporated.[22] In this study, the DCM was used as the organic solvent, as it had the properties of polarity, less toxicity, and low boiling point. Its solubility in water was 13 g/L at 20°C.

A higher volume ratio of the outer water phase to the organic phase improved the encapsulation as both the actual DL and EE increased with the volume ratio (Figure 3). At volume ratios of 30–80, EE increased gradually from 19.2 to 29.7% ($p < 0.05$). A further increase in the volume ratio led to no significant change in EE ($p > 0.05$). DL led to a remarkable increase in the volume ratio from 30–50 to 80 and 100 ($p < 0.05$). The surfaces of the NPs prepared at a high volume ratio were also much smoother compared to that prepared at a low ratio, as shown by the morphological examination with scanning electron microscopy (SEM).

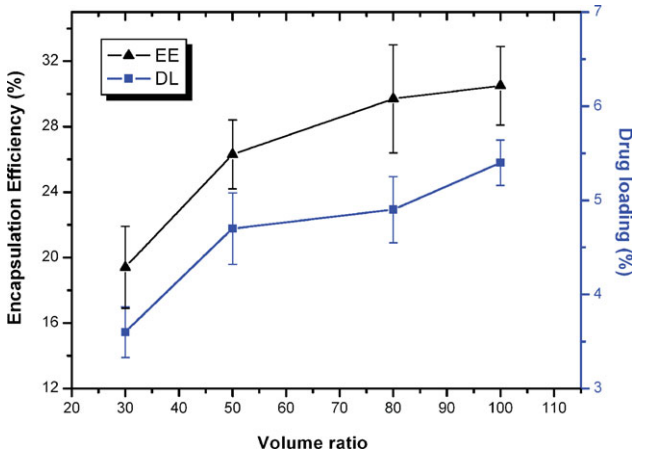

Figure 3. Volume ratio of the outer water phase to the organic phase on DL and EE (in the fabrication process of 5-FU loaded PLGA NPs). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

The double-emulsion process determined that a high volume of the outer water phase was required. After the formation of the double emulsion, the drugs in the first emulsion could travel to the outer water phase because of its hydrophilic properties because it had to be agitated for several hours to eliminate the organic solvent. If there was a small amount of outer water phase, the DCM could not be easily diffused, released, and then dissolved into water. A high volume of outer water phase could speed up solidification at a high volume ratio.

However, NPs prepared with a high volume ratio of the outer water phase to the first emulsion were larger in size (Table II), with a particle size ranging from 185.1 to 296.8 nm for volume ratios from 30 to 100. The increase in particle size was probably due to the reduction of the shearing force during the formation of the second emulsion and the homogenization process as a large outer water phase may have decreased the sonication efficiency when the probe sonicator was used during the first emulsion into the second one.

When NPs are smaller than 200 nm, they are more likely to penetrate and accumulate in cancerous cells.[9] In this particle size range, the drug-delivery vehicle can enter the tumor area on the basis of the enhanced permeability and retention effect.[23]

Table I. Effect of the TDL on the Properties of the Particles

TDL (% w/w)	Size (nm)	PDI	DL (%)	EE (%)
5	189.2 ± 1.5	0.190	2.4 ± 0.32	38.37 ± 2.4
10	205.4 ± 3.3	0.153	4.9 ± 0.28	29.69 ± 3.8
15	214.5 ± 2.5	0.124	6.3 ± 0.41	19.20 ± 2.7
20	233.6 ± 5.2	0.165	6.8 ± 0.35	17.13 ± 2.1

Table II. Effect of the Volume Ratio on the Properties of the NPs

Volume ratio	Particle size (nm ± SD)	PDI
30	185.1 ± 2.1	0.109
50	208.5 ± 1.8	0.142
80	265.1 ± 3.2	0.185
100	296.8 ± 4.9	0.131

Applied Polymer SCIENCE

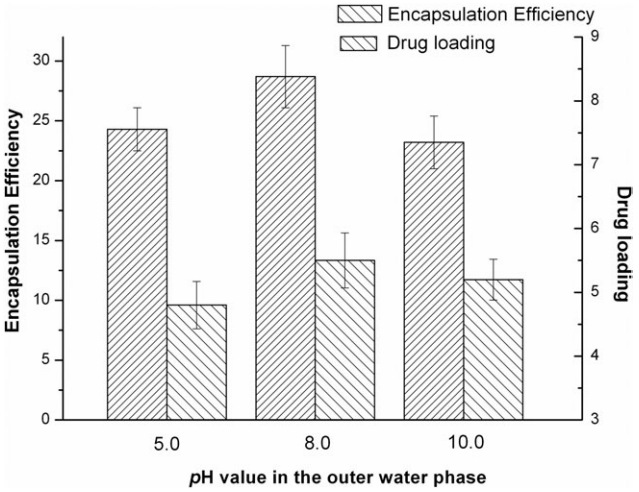

Figure 4. Influence of the pH values on DL and EE (in the fabrication process of the 5-FU-loaded PLGA NPs).

By considering the particle size (Table II) and DL and EE (Figure 3), we determined a volume ratio of the outer water phase to the organic phase of 50 to be the optimum as the size of the NPs was around 200 nm and relatively high DL and EE values were maintained.

pH Value of the Outer Aqueous Phase. The solubility of drugs in solution is normally pH dependent, and the drugs can easily diffuse into the outer water phase in the NP hardening process. When a drug is dissolved into a solution whose pH is at the drug's isoelectric point (pI), a minimum amount of the drug is dissolved.[24] Therefore, the adjustment of the pH value of the outer water phase to its pI is a potential method for increasing the DL. For the therapeutic drug 5-FU, the pI was 8.02, and the NPs prepared at a pH value of 8.0 of the outer water phase exhibited a higher DL and a higher EE compared to those prepared at other pH values (Figure 4) because a great repulsion was applied in the process and less drug appetency to the water occurred; this prevented the drug from being released from the first emulsion to the second one.

Optimized NPs

In light of the results derived from several fabrication parameters, the parameter effects were interactive. A compromise had to be made to get better combined results of DL, EE, and NP size. On the basis of the optimized fabrication parameters, the NPs were fabricated at a TDL of 10%, a volume ratio of the outer water phase to the organic phase of 50, and a pH value of the outer water phase of 8.0. The DL and EE of the NPs were 5.8 and 28.6%, respectively. This result shows the advantage of using the double-emulsion and solvent evaporation fabrication method and the adoption of optimized parameters in the preparation procedures. Niwa et al.[25] reported a similar formulation and finally obtained a highest DL of 2.65% and EE of 15.0%. McCarron et al.[12] demonstrated a similar 5-FU formulation using the emulsion polymerization technique and achieved a highest DL of 3.68% w/w. Therefore, the fabrication of 5-FU loaded PLGA NPs at specific optimized preparation parameters is necessary to get an enhanced DL and achieve a reasonable NP size.

The shape and surface morphology of the 5-FU loaded PLGA NPs was observed with SEM. The image in Figure 5(a) shows that the NPs appeared spherical with a relatively monodispersed size and nonporous surface. The average particle size was less than 200 nm. DLS was used to futher investigate the size and size distribution [Figure 5(b)]. They were tested at room temperature after the prepared samples were diluted 10 times with distilled water. Size calculations were done through intensity calculation. As shown in Figure 5(b), the *Z*-average particle size was 197.8 nm; this was in agreement with the SEM result. It also showed that the particles had a small PDI. When the NP size was between 70 and 200 nm, the particles are regarded as suitable for cancer treatment[9] and can be applied as passive targeting in cancer treatment.[26]

Eudragit S100 MSs Coated onto Core PLGA NPs

Eudragit S100 was coated onto the PLGA NPs to form MSs, and the ratio of coat to core was 5:1 w/w. The size of the MSs ranged from 30 to 50 μm in a monodisperse state, as shown by

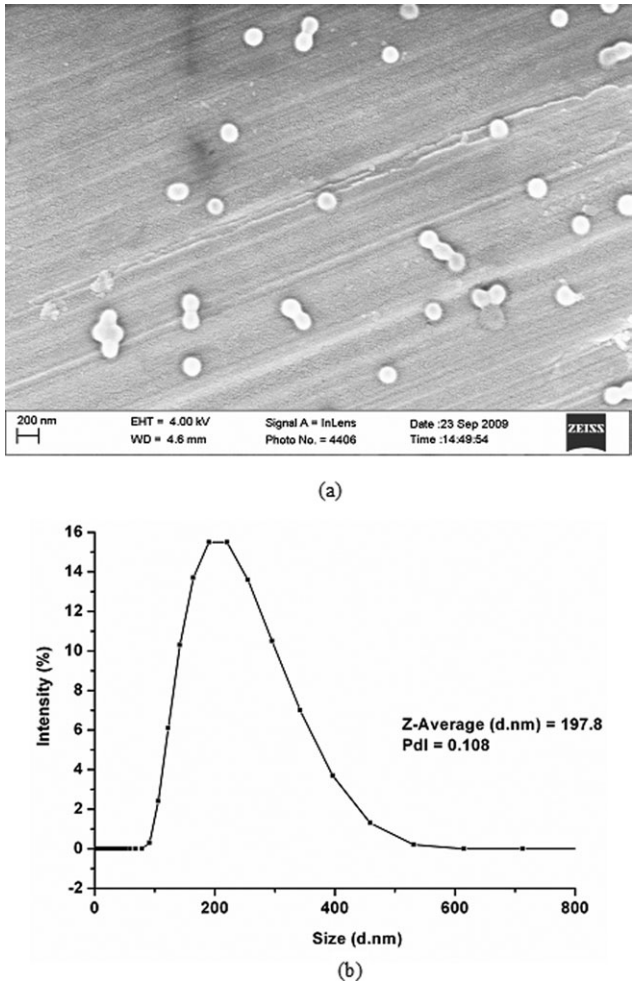

Figure 5. 5-FU-loaded PLGA NPs: (a) SEM image and (b) particle size distribution.

the SEM images (Figure 6). The MSs had rough surfaces and were spherical in shape. The image clearly showed that there were very fine particles on the surface of the MSs. These particles could have been the unentrapped PLGA NPs or the surfactant that had not been fully washed off in the final stage of the preparation.

Four drug-dissolution environments with an HCl buffer pH value of 1.2 and PBS pH values of 4.5, 6.8, and 7.4 were selected to mimic the real GIT. During the experiments, the MSs were held in the first three solutions for fixed periods of time (1, 2, and 2 h, respectively) to represent the different transit times in different parts of human body before the carrier reached the colon area. The NPs were then placed in the pH 7.4 PBS solution, and the test was not finished until the drug was fully dissolved.

The drug-release results show that nearly no drug was released in the first 3 h at pHs of 1.2 and 4.5 (Figure 7). This confirmed that the Eudragit S100 coated MSs were pH dependent[11] and the drug loaded NPs were perfectly encapsulated in the MS matrix. When the environment changed to a pH value of 6.8, which was used to mimic the small intestine, only a very limited amount of drug was released within 2 h, about 5.2% cumulative drug release.

The release of 5-FU from the PLGA NPs was then carried out in a PBS solution at pH 7.4; this was an environment similar to the colon and rectum. There was a burst release in the first few hours followed by an extended slow drug release of up to 120 h (Figure 7).

This unique and extended release profile could be further explored in targeted drug delivery, in which the drug-delivery carriers first recognize the tumor site and attach onto the cells[27] and, then, a sustained and delayed drug release can be achieved that will follow the pattern presented in Figure 7. Because of the swelling and erosion of the PLGA polymer, the release phases were characterized by pore diffusion in the initial phase and polymer erosion and degradation in the second phase.[28] In this way, drug release was controlled by these changes in the encapsulation materials, which led to a prolonged half-lifetime of the drug. Therefore, the results offer great potential for practical application in the treatment of colorectal cancer.

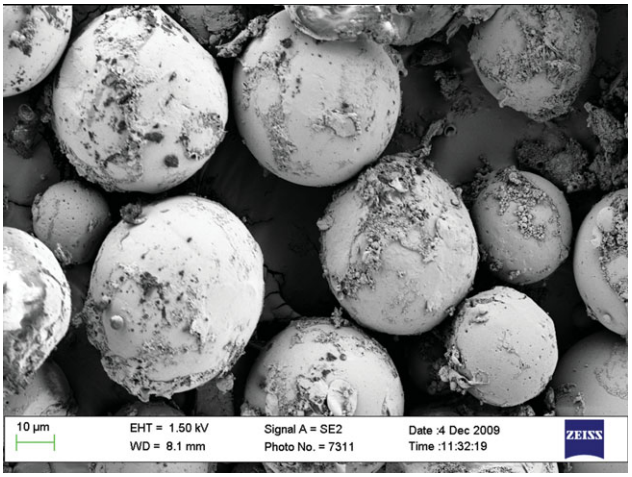

Figure 6. SEM image of the Eudragit-coated MSs (coating on 5-FU-loaded PLGA NPs). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

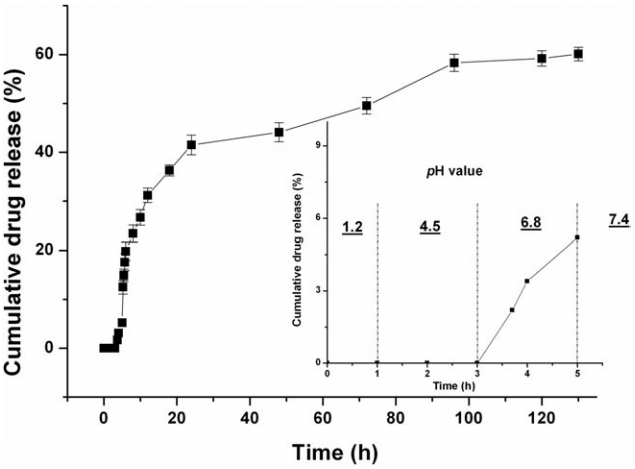

Figure 7. Dissolution profile of the PLGA NPs and Eudragit S 100 MSs (embedded picture).

CONCLUSIONS

In this study, pH-sensitive NPs with enhanced DLs were successfully fabricated by the microencapsulation of optimized 5-FU-loaded PLGA NPs. Enhanced DL, EE, and NP size were achieved by optimization of the nanofabrication parameters. Eudragit S100 MSs were able to retain the NP integrity and protect the embedded drug-loaded NPs to pass through the GIT with different pH values. Therefore, this kind of drug-delivery system has potential applications in the transport of therapeutic drugs to the colorectal area.

ACKNOWLEDGMENT

One of the authors (Y.W.) would like to express his gratitude for a Deakin University International Research Scholarships (DURIS) scholarship from Deakin University.

REFERENCES

1. Zhang, H.; Bei, J.; Wang, S. *J. Appl. Polym. Sci.* **2007**, *106*, 3757.
2. Qurratul, A.; Sharma, S.; Khuller, G. K.; Garg, S. K. *J. Antimicrob. Chemother.* **2003**, *51*, 931.
3. Asghar, L. F. A.; Chandran, S. *J. Pharm. Pharm. Sci.* **2006**, *9*, 327.
4. Naidu, B. V. K.; Paulson, A. T. *J. Appl. Polym. Sci.* **2011**, *121*, 3495.
5. Li, X. M.; Xu, Y. L.; Chen, G. G.; Wei, P.; Ping, Q. N., *Drug Dev. Ind. Pharm.* **2008**, *34*, 107.
6. Liu, F.; Park, J.-Y.; Zhang, Y.; Conwell, C.; Liu, Y.; Bathula, S. R.; Huang, L. *J. Pharm. Sci.* **2010**, *99*, 3542.

7. Sinha, R.; Kim, G. J.; Nie, S. M.; Shin, D. M. *Mol. Cancer Ther.* **2006**, *5*, 1909.
8. Khan, M. Z. I.; Prebeg, Z.; Kurjakovic, N. *J. Controlled Release* **1999**, *58*, 215.
9. Gaumet, M.; Vargas, A.; Gurny, R.; Delie, F. *Eur. J. Pharm. Biopharm.* **2008**, *69*, 1.
10. Govender, T.; Stolnik, S.; Garnett, M. C.; Illum, L.; Davis, S. S. *J. Controlled Release* **1999**, *57*, 171.
11. Domnina, Y. A.; Yeo, Y.; Tse, J. Y.; Bellas, E.; Kohane, D. S. *J. Biomed. Mater. Res. Part A* **2008**, *87*, 825.
12. McCarron, P. A.; Hall, M. *Int. J. Pharm.* **2008**, *348*, 115.
13. Zhang, Z. P.; Feng, S. S. *Biomaterials* **2006**, *27*, 4025.
14. Smeets, R.; Gerhards, F.; Stein, J.; Paz, R. M. P.; Vogt, S.; Pautke, C.; Weitz, J.; Kolk, A. *J. Biomed. Mater. Res. Part A* **2010**, *96*, 177.
15. Yoo, J.-W.; Giri, N.; Lee, C. H. *Int. J. Pharm.* **2011**, *403*, 262.
16. Vaidya, A.; Jain, A.; Khare, P.; Agrawal, R. K.; Jain, S. K. *J. Pharm. Sci.* **2009**, *98*, 4229.
17. Raffin, R. P.; Colome, L. M.; Pohlmann, A. R.; Guterres, S. S. *Eur. J. Pharm. Biopharm.* **2006**, *63*, 198.
18. Lim, D. W.; Park, T. G. *J. Appl. Polym. Sci.* **2000**, *75*, 1615.
19. Krishnamachari, Y.; Madan, P.; Lin, S. S. *Int. J. Pharm.* **2007**, *338*, 238.
20. Zhang, C.; Cheng, Y.; Qu, G. W.; Wu, X. L.; Ding, Y.; Cheng, Z. H.; Yu, L. L.; Ping, Q. N. *Carbohydr. Polym.* **2008**, *72*, 390.
21. Mao, S. R.; Shi, Y.; Li, L.; Xu, J.; Schaper, A.; Kissel, T. *Eur. J. Pharm. Biopharm.* **2008**, *68*, 214.
22. Bilati, U.; Allemann, E.; Doelker, E. *J. Microencapsul.* **2005**, *22*, 205.
23. Kunii, R.; Onishi, H.; Ueki, K. I.; Koyama, K. I.; Machida, Y. *Drug Delivery* **2008**, *15*, 3.
24. Leo, E.; Pecquet, S.; Rojas, J.; Couvreur, P.; Fattal, E. *J. Microencapsul.* **1998**, *15*, 421.
25. Niwa, T.; Takeuchi, H.; Hino, T.; Kunou, N.; Kawashima, Y. *J. Controlled Release* **1993**, *25*, 89.
26. Shi, X.; Du, Y.; Yang, J.; Zhang, B.; Sun, L. *J. Appl. Polym. Sci.* **2006**, *100*, 4689.
27. Li, P.; Wang, Y.; Zeng, F.; Chen, L.; Peng, Z.; Kong, L. X., *Carbohydrate Res* **2011**, *346*, 801.
28. Morlock, M.; Kissel, T.; Li, Y. X.; Koll, H.; Winter, G. *J. Controlled Release* **1998**, *56*, 105.