Formulation Optimization For High Drug Loading Colonic Drug Delivery Carrier

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Abstract—**High drug loading (DL) carrier is an effective way to cure the cancerous cells. High drug loading is also one of the key issues in the drug delivery research, especially the colonic drug delivery system by oral administration. The times of drug intake could be remarkably reduced if high drug loading carriers are administered. At the same time, the related formulation materials could be effectively utilized. One major obstacle with the preparation of this system is the difficulty to encapsulate the hydrophilic drug into hydrophobic encapsulation polymer. A design of high drug loading delivery system with biodegradable, biocompatible materials and optimization of the fabrication process is a potential solution to solve the problem. So in this research, 5-Fluorouracil (5-FU) loaded Poly (lactide-co-glycolide) (PLGA) nanoparticles were prepared by double emulsion and solvent evaporation method. Several fabrication parameters including theoretical drug loading, volume ratio of outer water phase to the first emulsion, *p*H value of outer aqueous phase and emulsifier PVA concentration were optimized to get a high drug loading nanoparticles.**

The result shows that with the increase of theoretical drug loading, the actual drug loading increased gradually. When adjusted the *p*H value of outer aqueous phase to the isoelectric point (8.02) of 5-Fluorouracil, the drug loading exhibited a higher one compared to other *p*H value solution. Relative higher volume ratio of outer water phase to the first emulsion was also beneficial for the enhancement of drug loading. But the nanoparticles size increased simultaneously due to the lower shearing force. When increased the PVA concentration, the drug loading showed an increase first and following a drop.

Keywords-5-Fluorouracil (5-FU); PLGA; optimization; nanoparticles

I. INTRODUCTION

Colorectal cancer (CRC) is the second cancer related death after lung cancer [1]. And Australia has one of the highest incidence rates of colorectal cancer in the world. Every year, lots of people are diagnosed with or die of this disease. The cancerous cells usually begin from non-cancerous cells called polyps, but from time to time, it becomes cancerous cells.

In recent years, the chemotherapy is more and more popular, as the traditional treatments like the surgery and radiation therapy hurt the patients a lot both in mental and physical. The chemotherapy is convenient to be administered and painless to the patients. Among the therapeutic drugs which have been put into practical use, 5-Fluorouracil (5-FU) is one of the most potent and popular ones. Since its introduction in 1950s, 5-FU still remains the only effective chemotherapeutic agent for the treatment of colorectal cancer (CRC) [2]. And using some biodegradable and biocompatible polymer like Poly (lactide-co-glycolide) (PLGA) to encapsulate the 5-FU into the nanoparticles could diminish the side-effect of 5-FU, such as short half life time and rapid plasma clearance. Also such a design to form drug delivery carrier could control the drug release by the degradation and erosion of PLGA.

The drug loading is one of the key issues in the process of such an encapsulation. The times of drug intake could be minimized if high drug loading carriers are administered. 5-FU is a hydrophilic drug, while the PLGA is a hydrophobic polymer. So this kind of encapsulation is not that easy. People have been using variety of methods to improve the drug loading for the drug delivery carrier. McCarron and co-worker substituted the 1-alkylcarbonyloxymethyl, prodrug of 5-FU, for the 5-FU [3], which results in the drug loading from 3.68% to 47.23%. Leo adjusted the *p*H value of external aqueous phase to isoelectric point of the drugs [4]. The result shows an obviously high protein encapsulation. Bodmeier et al found that the less hydrophilic organic solvent in the oil phase prevented the encapsulated hydrophilic drugs from releasing to the outer water phase [5].

In this research, different processing parameters including theoretical drug loading, *p*H value of outer aqueous phase, volume ratio of outer water phase to the first emulsion and PVA concentration were optimized for the poly (lactide-co-glycolide) (PLGA) nanoparticles of 5-FU.

II. MATERIALS AND METHODS

A. Materials

PLGA-COOH 50/50 with an average molecular weight of 40k was purchased from Ji'nan Jidai Biological Co. Ltd (Shandong, China). The surfactant used in the emulsification process was poly (vinyl alcohol) (PVA) with 86.7%-88.7% hydrolysis degree and molecular mass 31k (Sigma Chemical CO, USA). All the chemicals used were analytical grade and used without further purification.

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B. *Preparation of PLGA nanoparticles*

The PLGA nanoparticles loaded with 5-FU were prepared by a modified $W_1/O/W_2$ multiple emulsion and solvent evaporation technique (Figure 1) as per previous published paper [6]. Briefly, 100 mg of PLGA was dissolved in 6 ml methylene chloride (DCM). 5-FU was dissolved into a water solution which was adjusted concentration to certain value. Into the organic phase (O), certain amount of aqueous drug solution (W_1) was emulsified using probe sonicator for 2 minutes with a 30% of amplitude to form W_1/O emulsion. The primary emulsion was injected into an aqueous phase containing Poly (vinyl alcohol) (PVA) (external phase, W_2) and sonicated for 1 minutes. The resulting $W_1/O/W_2$ emulsion was stirred with a magnetic stirrer for 5 hours to allow the solvent evaporation and particle hardening. The nanoparticles were then separated by ultra-centrifugation at 12k rpm for 20 minutes and washed with distilled water for three times, in order to remove the 5-FU on the surface of particles and the excess of surfactant. The washing solution was eliminated by a further centrifugation. The solvent evaporation can also be conducted by rotary evaporator at a reduced pressure. Finally, the nanoparticles were collected from a freeze-drier and preserved in a desiccator until the time of evaluation.

C. *Drug loading(DL) and encapsulation efficiency(EE) of PLGA nanoparticles*

To determine the 5-FU amount entrapped in the PLGA nanoparticles, indirect method was carried out to test the content of the drug in the nanoparticles by measuring the drugs that were not encapsulated. The prepared nanoparticle solution was centrifuged at 12k rpm for 15 minutes. Then the supernatant was collected and tested by ultraviolet-visible (UV) spectrometer at a wavelength of 265 nm. The DL and EE be calculated in the following equations:

$$\text{Drug loading (DL)}/\% = (A-B)/C*100 \quad (1)$$

$$\text{Encapsulation efficiency (EE)}/\% = (A-B)/A*100 \quad (2)$$

A: total drugs

B: drug in supernatant

C: weight of nanoparticles

III. RESULTS AND DISCUSSION

A. *Effect of theoretical drug loading of 5-FU*

In order to encapsulate large amount of drugs into the nanoparticles and figure out how the theoretical drug loading affects the actual drug loading of the nanoparticles, four theoretical drug loading amounts 5%, 10%, 15% and 20% (w/w) were selected. From the table I, as the increase of theoretical drug loading, actual drug loading ranges from 2.4% to 6.8%. However, the corresponding drug encapsulation efficiency decreases from 27.58% to 17.13%. The reason for that is the stability of first emulsion was reduced when initial drug feeding increased. So if small amount of drugs were feeded in the fabrication process, the drug loading would be higher. But in this case, the encapsulation efficiency is a little low. The patients who take the drugs have to increase the times and frequency of drug administration, in order to keep enough drug concentration in the body. If high amount of drugs are feeded in the preparation, lot of drugs could not be encapsulated in and result in the loss of expensive drugs. So the compromise scheme should be retrieved. That is to get a relative high drug loading and decrease the manufacturing cost. 10% of the initial drug feeding seems to be the optimal formulation, as it keeps a balance of relative high drug loading and encapsulation efficiency. So in the following preparation, the 10% theoretical drug loading was used as the starting parameter.

TABLE I. EFFECT OF THEORETICAL DRUG LOADING ON THE PROPERTY OF THE PRODUCTS

TRL[a]	Size (nm)	PDI (poly-dispersity)	DL (%)	EE (%)
5%	189.2	0.190	2.4	27.58
10%	205.4	0.153	4.9	25.80
15%	214.5	0.124	6.3	19.20
20%	233.6	0.165	6.8	17.13

a. Theoretical drug loading

B. *Volume ratio of the outer water phase to first emulsion on the drug loading*

In the $W_1/O/W_2$ fabrication process, first emulsion is the dispersing phase; while the outer water phase is the continuous phase. For the double emulsion preparation method, the outer water phase is used for hardening the nanoparticles and promoting the organic solvent to be evaporated. In this research, we used the DCM as the organic solvent, as it has the properties of polarity, less toxicity and low boiling point. And

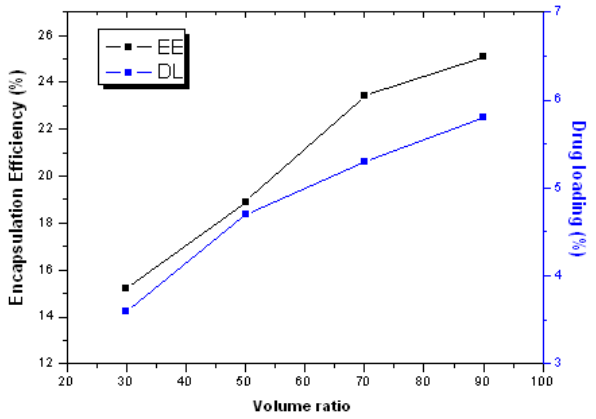

Figure 1. Volume ratio of the outer water phase to first emulsion on drug loading and encapsulation efficiency

its solubility in water is 13 g/L at 20°C [7]. With the increase of this volume ratio, the actual drug loading increased and the surface of the nanoparticles prepared at a high volume ratio was much smoother compared to that prepared at a low ratio one. The reason is as follows, after the formation of the double emulsion, it has to be agitated for several hours in order to eliminate the organic solvent. If there is a small amount of outer water phase, the DCM could not be easily diffused and released out and then be dissolved into water. This results in

particles forming a longer time, which the drugs in the first emulsion could travel to outer water phase due to its hydrophilic property. If the volume of outer water phase is higher, that could speed up the solidification time at high volume ratio of the outer water phase to first emulsion. At the same time, we found that the nanoparticles prepared by the high volume ratio have larger size than that of the low volume ratio one. The probe sonicator was performed when the first emulsion was emulsified into the second emulsion. The large outer water phase may decrease the sonication efficiency. So the increase in the particle size was probably attributed to reduction of the shearing force during the formation of second emulsion and the homogenization process.

TABLE II. EFFECT OF VOLUME RATIO ON THE PROPERTY OF THE PRODUCT

Volume ratio	Particle size (nm)	PDI (polydispersity)
30	185.1	0.109
50	203.4	0.175
70	229.5	0.171
90	258.7	0.102

C. *pH value of outer aqueous phase*

Normally the solubility of drug is *p*H dependent. And when a drug is dissolved into a solution whose *p*H adjusts to the drug's isoelectric point, fewer drugs would be dissolved into the solution [4]. For the 5-FU, the isoelectric point is 8.02. In order to compare the effect of *p*H in the outer aqueous phase, three different *p*H values 5.0, 8.02 and 10.0 were selected. From the figure 2, we could clearly see that the products prepared by 8.02 *p*H value of outer water phase exhibit a high drug loading. During the process of nano-fabrication, when we got the first emulsion and kept further emulsifying in the outer water phase, the drugs in the first emulsion could be easily released out due to its appetency to aqueous phase. By altering the outer aqueous phase *p*H value to the isoelectric point of

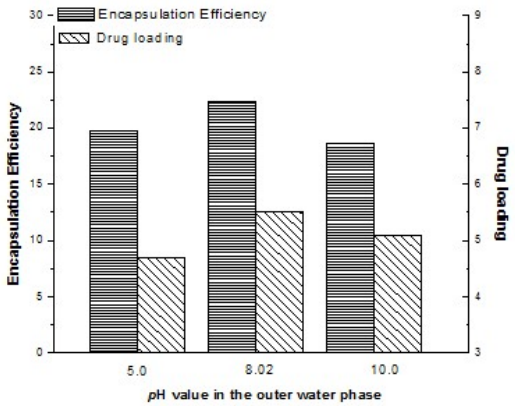

Figure 2. Influence of pH value on drug loading and encapsulation efficiency

drugs could obviously reduce the drug loss, as the drugs have the minimum soluble in that *p*H value solution.

D. *PVA concentration*

The PVA located between the oil phase and the water phase to stabilize the formed nano-emulsion and prevent the aggregation. The nanoparticles were formed with the help of PVA to stabilize the emulsion solvent and droplets formation. Different PVA concentrations were used in the sonication and homogenization process, in order to study the external aqueous phase on the nanoparticles properties. 0.2%, 0.6% and 1.2% (w/v) concentration PVA were selected to do the experiments.

The prepared nanoparticles with higher concentration PVA exhibited a slight higher particle size than the one with lower concentration. The larger particle size of nanoparicles with higher surfactant is probably due to the adsorption of surfactant to the particle surface. Although repeated washings were conducted, some of the adsorbed PVA were not washed away.

TABLE III. INFLUENCE OF PVA CONCENTRATION ON PARTICLE SIZE AND DRUG LOADING

concentration[a]	Particle size (nm)	PDI (poly-dispersity)	DL (%)	EE (%)
0.2%	185	0.163	5.2	22.3
0.6%	204	0.110	5.6	25.8
1.2%	237	0.145	4.2	19.6

a. PVA concentration (w/v)

From the table III, when the PVA concentration increases to 1.2% (w/v), the drug loading dropped dramatically. This is probably ascribed to the residue PVA on the particle surface. Based on the equation 1, if the residue existed in the final product, the addition of the PVA weight was ignored, which result in the lower calculation of the drug loading.

CONCLUSIONS

In this research, 5-FU loaded PLGA nanoparticles were prepeared by the double emulsion and solvent evaporation method. Different processing parameters were optimized to get a high drug loading carrier. Results indicated that the particles prepared by high theoretical drug loading got a high actual drug loading, while the corresponding encapsulation efficiency was a little low. Adjusting *p*H of the outer water phase to the isoelectric point of the drug and high volume ratio of outer water phase to first emulsion could enhance the drug loading. Proper amount of PVA concentration was beneficial to increase the drug loading.

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REFERENCE

[1] Lamprecht, A., Yamamoto, H., Takeuchi, H. & Kawashima, Y. (2003) Microsphere design for the colonic delivery of 5-fluorouracil. Journal of Controlled Release, 90, 313-322.

[2] Lin, F. H., Lee, Y. H., Jian, C. H., Wong, J. M., Shieh, M. J. & Wang, C. Y. (2002) A study of purified montmorillonite intercalated with 5-fluorouracil as drug carrier. Biomaterials, 23, 1981-1987.

[3] McCarron, P. A. & Hall, M. (2008) Incorporation of novel 1-alkylcarbonyloxymethyl prodrugs of 5-fluorouracil into poly(lactide-co-glycolide) nanoparticles. International Journal of Pharmaceutics, 348**,** 115-124.

[4] Leo, E., Pecquet, S., Rojas, J., Couvreur, P. & Fattal, E. (1998) Changing the pH of the external aqueous phase may modulate protein entrapment and delivery from poly(lactide-co-glycolide) microspheres prepared by a w/o/w solvent evaporation method. Journal of Microencapsulation, 15**,** 421-430.

[5] Bodmeier, R. & McGinity, J. W. (1988) Solvent selection in the preparation of poly(DL-lactide) microspheres prepared by the solvent evaporation method. International Journal of Pharmaceutics, 43**,** 179-186.

[6] Gryparis, E. C., Mattheolabakis, G., Bikiaris, D. & Avgoustakis, K. (2007) Effect of conditions of preparation on the size and encapsulation properties of PLGA-mPEG nanoparticles of cisplatin. Drug Delivery, 14**,** 371-380.

[7] http://en.wikipedia.org/wiki/Dichloromethane