Optimized preparation of γ-polyglutamic acid/chitosan nanocapsule

X Ma[1,2], M M Zhang[1], S W Chen[3*]

[1] *School of Perfume and Aroma Technology, Shanghai Institute of Technology, Shanghai- China*

[2] *Bright Dairy & Food Co. Ltd, Shanghai- China*

[3] *Shanghai Jiaotong University affiliated Sixth people's hospital*

Received 1 March 2014, www.tsi.lv

Abstract

This paper described the preparation of novel biodegradable nanocapsule based on self-assembly of γ-Polyglutamic acid (γ-PGA) and chitosan (CS). After the Plackett-Burman design (PBD), the impact of mass concentration and volume of γ-PGA and pH value of CS were characterized by size and PDI of the nanocapsule. A Box-Behnken design (BBD) was used to optimize the preparation of the nanocapsule. The optimized condition was: pH value of CS was 4.0; volume of γ-PGA was 18mL; mass concentration of γ-PGA was 0.1g/L. The Z-Ave and PDI of the nanocapsules prepared under the best conditions were 175 nm and 0.15 respectively. In this work, we have shown that nano-sized particles have been successfully assembled from the γ-PGA and chitosan without employing covalent linkages between these biopolymers. These results will provide a novel concept in the design of carrier systems composed of polyion complex (PIC).

Keywords: γ-Polyglutamic acid, chitosan, nanocapsule, optimization, response surface methodology

1 Introduction

γ-Polyglutamic acid (γ-PGA) is an anionic polymer which consists of D-or L-glutamic acid via α-amino and γ-carboxyl group to form γ-glutamyl bond (Figure 1). As a water-soluble biodegradable polymeric material, it is widely used because of the edible, non-toxic, cohesiveness and other characteristics. In the current study reports, γ-PGA is mainly used as drug carriers [1], bio-adhesive in high value-added field of medicine. What's more, γ-PGA can be used as flocculants, heavy metal chelating agent for water treatment [2], it can also serve as moisture holding agent, antifreeze, preservatives used in fruits, food, vegetables, health products, cosmetics and so on. There are a large number of free carboxyl groups in the γ-PGA molecular chain which can be modified by chitosan [3], polyethylene glycol [4], L-phenylalanine [5] and so on. The modified parts of γ-PGA could be as drug carriers such as amoxicillin [6], insulin [7]. What is more, γ-PGA can also be drug carriers direct binding with camptothecin [8], paclitaxel [9], penicillamine [10] and so on. As a new, safe and harmless biological adhesive γ-PGA can also be used to control continuous bleeding in tissue and repair aortic cutting.

Chitosan (CS) whose active group was amino was a deacetylated chitin product, containing β-[1→4]-linked 2-acetamido-2-deoxy-D-glucopyranose and 2-amino-2-deoxy-D-glucopyranose units (Figure 2). Because of these amino groups in the molecular structure, CS is a weak base and the only natural cationic polymers. CS has not only good biocompatibility, reliability safety and low immunogenicity, but also hypolipidemic, nor cholesterol, anti-bacterial and increasing immune physiological activities. As a good natural bio-medical material, CS can be used as wound dressing materials [11] and drug carries [12, 13].

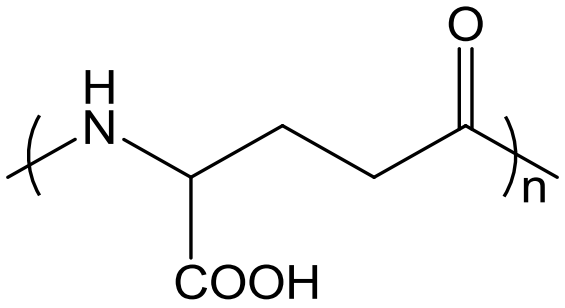

FIGURE 1 The molecular structure of γ-PGA

The response surface methodology (RSM) design was one of experimental design methods which could find improved or optimal process settings [14, 15]. The purpose of our research was:

1) The critical factors that affect the size of γ-PGA/CS nanocapsules were picked via a Plackett-Burman design (PBD);
2) The preparation conditions of γ-PGA/CS nanocapsules was optimized via a Box-Behnken design (BBD).

* ***Corresponding author*** e-mail: chenshiven@126.com

2 Materials and methods

2.1 MATERIALS

γ-Polyglutamic acid (γ-PGA) was prepared in our laboratory using the biosynthetic methods. γ-PGA was purified as follows: the crude products were dissolved in distilled water to give a mass concentration of 2% and the dialyzed against distilled water for 24h. The dialysate was lyophilized in form of white powder and used for further experiments. CS was purchased from Shanghai Plus Bio-Sci&Tech Co., Ltd. (Shanghai, China) (Grade: BR) with molecular weight of ~150,000 and 90% degree of deacetylation. The other reagents were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). The γ-PGA aqueous solution was prepared with different concentration. The solution of CS was prepared with different concentration in aqueous solution with 1% acetic acid. The water used in the experiments is all distilled. 20% NaOH aqueous solution and 2mol/L HCl were used to adjust the pH of the γ-PGA aqueous solution and CS solution.

2.2 PREPARATION OF CS/γ-PGA NANOCAPSULE

γ-PGA solution and CS solution were used for preparation of γ-PGA/CS nanocapsule. The preparation technique based on the self-assembly of polyelectrolytes, in which the anion polymer (PGA) interacts with the cation polymer (CS) at normal temperature and pressure. The preparation methods were as follow: the γ-PGA solution was dropwise added into the CS solution under continuous stirring; the mixture was further stirred for 60 min at ambient temperature after adding γ-PGA solution. The CS/γ-PGA nanocapsules were obtained by lyophilization of the mixture.

The experimental design (Table 2) was contrived based on Design-Expert 7.0. Z-Ave was the response variable. After data processing by Design-Expert 7.0, A (pH value of CS), D (Mass concentration of γ-PGA) and J (Volume of γ-PGA) were selected for further analysis by Box-Behnken. The optimum preparation conditions selected by PBD were used for further analysis by BBD.

3.2 BOX-BEHNKEN DESIGN (BBD)

As one design method of RSM, BBD was used to optimize the preparation conditions of γ-PGA/CS nanocapsule. Three factors selected by PBD were tested in this design. Each factor was tested at 3 levels (-1, 0 and 1, Table 3). The experimental design (Table 4) was contrived based on Design-Expert 7.0. All experiments were performed in triplicate. Average size (Z- Ave) and polydispersity index (PDI) were the response variables.

2.3 SIZE AND ZETA-POTENTIAL MEASUREMENTS

The size and Zeta-potential of the nanocapsules were measured at 25 °C by a Malvern zetasizer Nano-ZS instrument (Malvern Instruments Ltd.). Each sample was measured three times and average serial data were calculated.

3 Optimization by RSM

3.1 PBD

PBD is an experimental design method for two-level and the most popular fractional design. It is suitable for optimizing multitudinous factors, since it makes it possible to pick up the relevant factors from a long list (16).

In the process of preparation conditions optimization, 9 factors (Table 1) were tested. Each factor was tested at both low (–1) and high (1) levels (Table 1).

TABLE 1 Levels and experimental factors of Plackett-Burman design

Code	Name	Unit	Levels	
			Low level (-1)	High level (1)
A	pH value of CS	/	3.0	6.0
B	pH value of γ-PGA	/	6.0	7.4
C	Mass concentration of CS	g/L	0.20	1.00
D	Mass concentration of γ-PGA	g/L	0.20	0.40
E	Stirring speed	r/min	50	150
F	Concentration of Mg2+	mol/L	0.002	0.006
G	Reaction time after dropping	min	10	60
H	Dropping speed	mL/h	0.10	1.00
J	Volume of γ-PGA	mL	2	10

TABLE 2 Plackett-Burman experiment design and response values

Std No	Run	A	B	C	D	E	F	G	H	J	K	L	Z-Ave/nm
1	13	1	1	-1	1	1	1	-1	-1	-1	1	-1	793.0
2	5	-1	1	1	-1	1	1	1	-1	-1	-1	1	179.0
3	10	1	-1	1	1	-1	1	1	1	-1	-1	-1	779.0
4	1	-1	1	-1	1	1	-1	1	1	1	-1	-1	300.5
5	3	-1	-1	1	-1	1	1	-1	1	1	1	-1	219.5
6	15	-1	-1	-1	1	-1	1	1	-1	1	1	1	263.5
7	14	1	-1	-1	-1	1	-1	1	1	-1	1	1	170.0
8	4	1	1	-1	-1	-1	1	-1	1	1	-1	1	206.7
9	9	1	1	1	-1	-1	-1	1	-1	1	1	-1	190.0
10	11	-1	1	1	1	-1	-1	-1	1	-1	1	1	738.5
11	7	1	-1	1	1	1	-1	-1	-1	1	-1	1	433.0
12	12	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	150.5
13	8	0	0	0	0	0	0	0	0	0	0	0	197.0
14	6	0	0	0	0	0	0	0	0	0	0	0	193.5
15	2	0	0	0	0	0	0	0	0	0	0	0	190.0

TABLE 3 Levels and experimental factors of BBD

Factors	Code	Level		
		-1	**0**	**1**
pH value of CS	X1	3.0	4.5	6.0
Volume of γ-PGA(mL)	X2	5	10	15
Mass concentration of γ-PGA (g/L)	X3	0.2	0.4	0.6

Table 4 Box-Behnken experiment design and response values

No.	Run	X1	X2	X3	Y1	Y2
1	1	-1	-1	0	340.5	0.2895
2	14	1	-1	0	213.5	0.1425
3	6	-1	1	0	326.0	0.2365
4	11	1	1	0	381.0	0.2805
5	2	-1	0	-1	293.5	0.4065
6	15	1	0	-1	233.5	0.1845
7	13	-1	0	1	367.5	0.2220
8	7	1	0	1	366.0	0.3925
9	9	0	-1	-1	238.5	0.3475
10	3	0	1	-1	224.5	0.2685
11	10	0	-1	1	295.5	0.2055
12	4	0	1	1	327.0	0.2045
13	12	0	0	0	271.0	0.2325
14	5	0	0	0	281.0	0.2020
15	8	0	0	0	305.5	0.2255

Using the Design-Expert 7.0, the analysis, the results and the second-order empirical model of each factor on the responses were obtained.

$$Y_1 = \alpha_0 + \sum_{i=1}^{k} \alpha_i X_i + \sum_{i=1}^{j-1} \sum_{j=1}^{k} \alpha_{ij} X_i X_j + \sum_{i=1}^{k} \alpha_{ii} X_i^{\,2}, \; i \neq j \quad (1)$$

$$Y_2 = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{j-1} \sum_{j=1}^{k} \beta_{ij} X_i X_j + \sum_{i=1}^{k} \beta_{ii} X_i^{\,2}, \; i \neq j \quad (2)$$

where Y1 is the response value that is representative of Z-Ave; Y2 is the response value that was representative of PDI; α_0, α_i, α_{ij}, α_{ii}, β_0, β_i, β_{ii}, β_{ij} are the regression coefficients, Xi represent the variables of the system.

4 Results and discussion

4.1 FACTORS CHOSEN IN PBD

Many factors can affect the final results in preparation condition of γ-PGA/CS nanocapsule. In this research, 9 factors were chosen to estimate the relative variables. As polyelectrolytes γ-PGA and CS were different ionization in different pH value, so the pH value of solution might have an impact on the size of the nanocapsule. In the design factors A to D were the pH value and mass concentration of the two materials. In our previous work, we found that flocculation occurred between γ-PGA and CS if the concentration and the volume of two materials were not suitable. Therefore, except for the reaction conditions such as stirring speed, concentration of Mg^{2+}, reaction time after dropping and dropping speed the volume of γ-PGA was one of the factors. At the same time, the total volume of each sample was 20ml.

4.2 PBD RESULTS

To evaluate the quality of the model, an F-value test was conducted. The ANOVA of PBD is presented in Table 5. There is only a 1.43% chance that a "Model F-Value" this large could occur due to noise. Therefore, the model is significant and its R-Squared is 0.9645, that has to say the results are suitable for the experiment design and the further optimization. In this case D (Mass concentration of γ-PGA) and J (Volume of γ-PGA) are significant model terms, and B (pH value of γ-PGA), E (Stirring speed), F (Concentration of Mg2+), H (Dropping speed) are insignificant model terms. We chose pH value of CS, Mass concentration of γ-PGA and Volume of γ-PGA for further optimization. In the next experiments variables E and F took intermediate values, C, G and H took the high levels, while pH value of γ-PGA was natural value.

TABLE 5 The ANOVA of PBD

Source	Sum of Squares	df	Mean Square	F value	p-value Prob>F
Model	683387.5	9	75931.95	12.083580	0.0143
A	43224	1	43224.00	6.878539	0.0586
B	12818.4	1	12818.40	2.039882	0.2264
C	35730.25	1	35730.25	5.686006	0.0756
D	400332.3	1	400332.30	63.707680	0.0013
E	4531.853	1	4531.85	0.721186	0.4436
F	17495.6	1	17495.60	2.784198	0.1705
G	36212.05	1	36212.05	5.762678	0.0743
H	13682.25	1	13682.25	2.177353	0.2141
J	119360.9	1	119360.90	18.994730	0.0121
Curvature	73584.02	1	73584.02	11.709940	0.0267
Residual	25135.57	4	6283.89		
Lack of Fit	25111.07	2	12555.54	1024.942	0.0010
Pure Error	24.5	2	12.25		
Cor Total	782107.1	14			

4.3 ANOVA OF BBD

Based on the PBD, three factors (pH value of CS, Mass concentration of γ-PGA and volume of γ-PGA) significantly influenced the Z-Ave of the γ-PGA/CS nanocapsule. To define the optimum settings of these factor levels, a BBD with 15 experiments (Table 4) was used to estimate the model coefficients. The experimental points are located in the middle of a cube's edges (12 experiments, which used to factorial analysis) and at the centre of the cube. This ensured that independent estimates of the model's parameters were obtained. Regression analysis was used to estimate the regression coefficients of the model, each response can be described by a second-order empirical model, which is adequate for predicting the response in the experimental region.

A statistical test of the model fit was performed by comparing the variance due to the lack of fit with the pure error variance using the F-test. The Z-Ave analysis process of BBD is presented in Table 6. The Model F-value of 6.55 implies the model is significant. There is only a 2.61% chance that a "Model F-Value" this large could occur due to noise. In this case X3, X1* X2 and X1^2 are significant model terms. The "Lack of Fit F-value" of 2.73 implies the Lack of Fit is not significant relative to the pure error. There is a 27.91% chance that a "Lack of Fit F-value" this large could occur due to noise.

Three factors affect the Z-Ave of γ-PGA/CS nanocapsule in this order: X3> X2>X1, which matches with the result of PBD.

TABLE 6 The Z-Ave ANOVA of BBD

Source	SS	df	MS	F value	p-value Prob>F
Model	37901.13	9	4211.24	6.55	0.0261
X1	2227.78	1	2227.78	3.46	0.1218
X2	3633.78	1	3633.78	5.65	0.0634
X3	16744.50	1	16744.50	26.04	0.0038
X1* X2	8281.00	1	8281.00	12.88	0.0157
X1* X2	855.56	1	855.56	1.33	0.3009
X2* X3	517.56	1	517.56	0.80	0.4107
X1^2	4941.56	1	4941.56	7.68	0.0393
X2^2	189.64	1	189.64	0.29	0.6104
X3^2	196.31	1	196.31	0.31	0.6044
Residual	3215.10	5	643.02		
Lack of Fit	2584.94	3	861.65	2.73	0.2791
Pure Error	630.17	2	315.08		
Cor Total	41116.23	14			

The quadratic model obtained by regression analysis showed as follow:

$$Y_1 = 285.83 - 16.69 * X_1 + 21.31 * X_2 + 45.75 * X_3 + 45.50 * X_1 * X_2 + 14.63 * X_1 * X_3 + 11.38 * X_2 * X_3 + 36.58 * X_1 * X_1 - 7.17 * X_2 * X_2 - 7.29 * X_3 * X_3$$

The R-squared and Adj R-squared of this model are 0.9218 amd 0.7811, respectively. Therefore, there are insignificant terms in the model, this match with the ANOVA results. The C.V. % of 8.52 show that the model variation of the measured values is small; there is only 8.52% variability of the data. Moreover, Adeq Precision measures the signal-to-noise ratio, which value greater than 4 is desirable. The Adeq Precision of 7.230 in this model indicates an adequate signal (17).

The fit testing of regression model was shown in Figure 3. The Figure 3(a) showed that the normal plot of residuals was almost a straight line, indicating the potential of error distribution is normal. Predicted values and residuals showed irregular distribution as shown in the Figure 3(b), this matched with the residuals should be amorphous in the model fitting. Thus, the regression assumptions were reasonable, and fitting the regression model was appropriate. In sum, the model can be used to navigate the design space and has a certain degree of predictability.

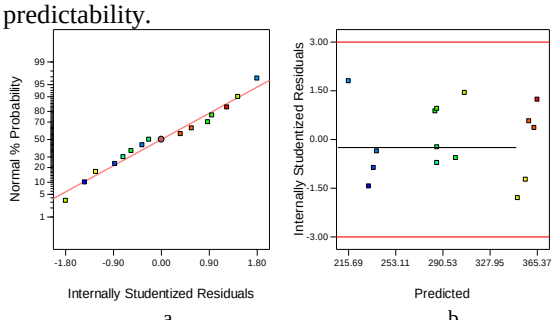

FIGURE 3 Regression model fit testing:
a) Normal Plot of Residuals; b) Residuals vs. Predicted

In the PDI analysis process, the quadratic model F-value of 3.58 and p-value Prob>F of 0.0868 imply the model is insignificant. Therefore, we chose the modified quadratic model in which the insignificant item X2 was removed. The PDI analysis process of BBD is presented in Table 6. The model F-value of 4.84 and p-value Prob>F of 0.0353 imply the model is significant. In this case, there are many insignificant model terms and only X1* X3 is significant model terms. The modified quadratic model is as follow:

$$Y_2 = 0.22 - 0.019 * X_1 - 0.023 * X_3 + 0.048 * X_1 * X_2 + 0.098 * X_1 * X_3 + 0.019 * X_2 * X_3 + 0.031 * X_1 * X_1 - 0.014 * X_2 * X_2 + 0.050 * X_3 * X_3$$

The R-squared and Adj R-squared of this model are 0.8657 and 0.6867, respectively. At the same time, the C.V. % and Adeq Precision are 16.60 and 8.247. Based on the data process of PDI, we can conclude that second-order model is not most adequate for describing the preparation conditions of γ-PGA/CS nanocapsule.

TABLE 7 PDI ANOVA of BBD

Source	SS	df	MS	F value	p-value Prob>F
Model	0.069833	8	0.008729	4.835171	0.0353
X1	0.002984	1	0.002984	1.652750	0.2460
X3	0.004163	1	0.004163	2.306088	0.1797
X1* X2	0.00912	1	0.009120	5.051808	0.0657
X1* X3	0.038514	1	0.038514	21.333370	0.0036
X2* X3	0.001521	1	0.001521	0.842499	0.3941
X1^2	0.003563	1	0.003563	1.973380	0.2097
X2^2	0.000704	1	0.000704	0.390196	0.5552
X3^2	0.009347	1	0.009347	5.177138	0.0632
Residual	0.010832	6	0.001805		
Lack of Fit	0.010322	4	0.002580	10.109270	0.0920
Pure Error	0.000511	2	0.000255		
Cor Total	0.080665	14			

4.4 OPTIMIZING RESULTS BY RSM

The effect of three factors on the size of the γ-PGA/CS nanocapsules was further analysed using contour and 3D response surface plots, which were the graphical representations of the regression model. By simulating the experimental results using the empirical model, these plots (Figure 4) efficiently identified the optimum values of the variables.

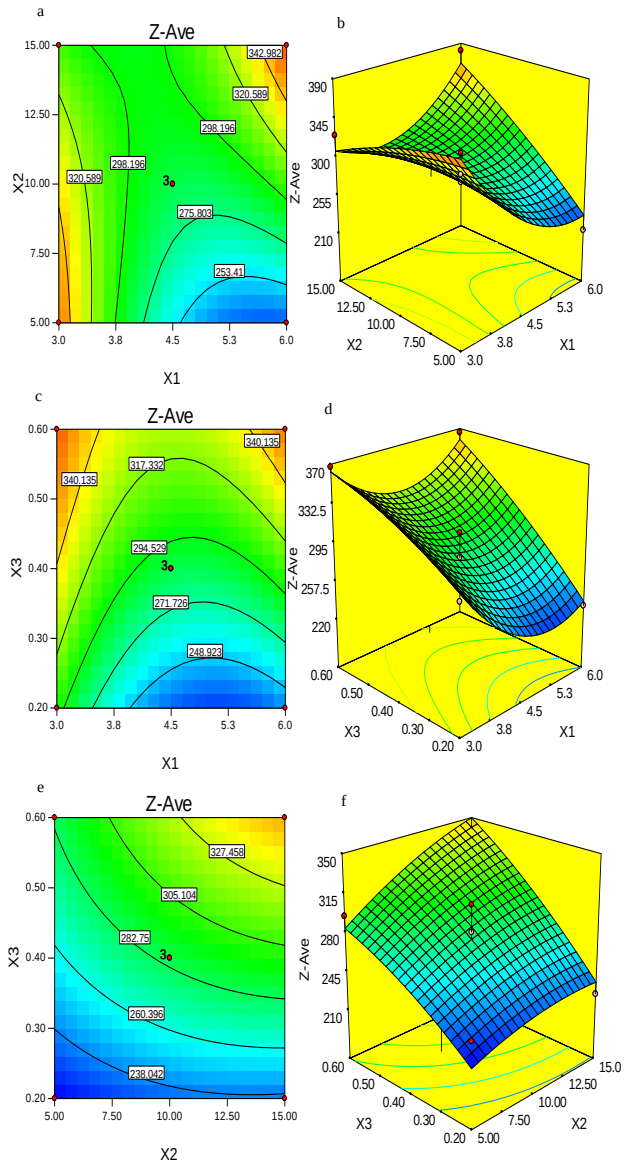

FIGURE 4 The contour and 3D response surface plots of two factors with the other factor at central levels; X1, X2, X3 represent pH value of CS, Volume of γ-PGA and Mass concentration of γ-PGA, respectively

Then it was convenient to understand the interactions between any two factors. From the Figure 4(a, b), we can know that pH value of CS and volume of γ-PGA had significant interaction. The Figure 4(c, d) showed pH value of CS and mass concentration of γ-PGA also had interaction. However, the interaction between volume of γ-PGA and mass concentration of γ-PGA was not significant (Figure 4(e, f)), the reason of that might be as follow: both of the two factors were affecting the total amount of γ-PGA.

4.5 VERIFICATION EXPERIMENT

After optimization, one set of experimental conditions with 4.0 of X1, 18ml of X2 and 0.1 of X3 was used as experimental conditions. The experimental results were listed in Table 7; the reproducibility and stability were very good (see Table 8). The size distribution of No.1 was showed in Figure5.

TABLE 8 Verification experiment results

No.	1	2	3
Z-Ave/nm	177.5	174.4	173.1
PDI	0.169	0.130	0.150

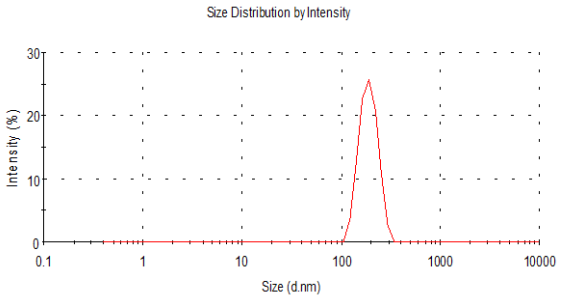

FIGURE 5 The size distribution of No.1

5 Conclusions

In this work, nanocapsules were successfully self-assembled from the γ-PGA and CS. PBD and BBD were used to optimize the preparation conditions. The optimized preparation conditions were that the pH value of CS was 4.0, the pH value of γ-PGA was natural pH value, the mass concentration of CS was 1g/L, the mass concentration of γ-PGA was 0.1g/L, the stirring speed was 100r/min, the concentration of Mg2+ was 0.004 mol/L, the reaction time after dropping was 60min, the dropping speed was 0.55 mL/h and the volume of γ-PGA was 18mL, in which the size of nanocapsule was 175 nm and its PDI was 0.15, it can used as drug carrier or flavour carrier.

Acknowledgment

The authors gratefully acknowledge the financial support provided by Science and Technology Commission of Shanghai, China (No. 10540501100& No.LM201343) and by the Open Project Program of State Key Laboratory of Dairy Biotechnology, Bright Dairy & Food Co. Ltd., (No. SKLDB2013-09).

References

[1] Akagi T, Watanabe K, Kim H, Akashi M 2010 *Langmuir* **26** 2406–13
[2] Bhatt R, Vries P, Klein JP, Tulinsky J, Lewis RA, Jack W, Singer J W 2007 *Polyglutamic acid-camptothecin conjugates and methods of preparation* USP: 7173041
[3] Chang C H, Lin Y H, Yeh C L, Chen Y C, Chiou SF, Hsu Y M, Chen YS, Wang C C 2010 *Biomacromolecules* **11** 133–42
[4] Ljubimova J Y, Black K L, Ljubimov A V, Eggehard H 2008 *Multifunctional Pharmaceutical Nanocarriers* **4** 233–62
[5] Keresztessy Z, Bodnár M, Ber E, Hajdu I, Zhang M, Hartmann JF, Minko F, Borbély, J 2009 *Colloid & Polymer Science* **287** 759–65

[6] Kurtaran H, Erzurumlu T 2006 *The International Journal of Advanced Manufacturing Technology* **27** 468–72
[7] Mi F L, Wu Y Y, Lin Y H, Sonaje K, Ho Y C, Chen C T, Juang J H, Sung H W 2008 *Bioconjugate Chemistry* **19** 1248–55
[8] Montgomery D C 2009 *Design and analysis of experiments*. Beijing, China: Posts Telecom Press
[9] Prencipe G, Tabakman S M, Welsher K, Liu Z, Goodwin A P, Zhang L, Henry J, Dai H J 2009 *Journal of the American Chemical Society* **131** 4783–7
[10] Sonaje K, Chen Y J, Chen H L, Wey S P, Juang J H, Nguyen H N, Hsu C W, Lin K J, Sung H W 2010 *Biomaterials* **31** 3384–94
[11] Tong R, Christian D A, Tang L, Cabral H, Baker J R Jr, Kataoka K, Discher D, Cheng J 2009 *Nanopolymeric therapeutics MRS Bulletin* **34** 422–31
[12] Tsao C T, Chang C H, Lin Y Y, Wu M F, Young T H, Han J L, Hsieh K H 2011 *Carbohydrate Polymers* **84** 812–9
[13] Wadhwa S, Mumper R J 2010 *Molecular pharmaceutics* **7** 854–62
[14] Xu X H, He M Z 2010 *Experimental design and application of design-expert* SPSS. Beijing, China: Science Press 36-59
[15] Yan S F, Zhu J, Wang Z C, Yin J 2011 *European Journal of Pharmaceutics and Biopharmaceutics* **78** 336–45
[16] Yao J, Xu H, Wang J 2007 *J Biomater Sci Polymer Edn* **18** 193–204
[17] Zhang H, Jiang Z, Guo C 2009 *The International Journal of Advanced Manufacturing Technology* **41** 110–21

Authors

Ma Xia, born in May 29, 1971, Shandong, China

Current position: Doctor of Fermentation Engineering, professor and master's supervisor in Shanghai institute of technology
University studies: Fermentation engineering in Tianjin University of Science & technology
Scientific interest: Biomaterials biosynthesis & applications fermentation of function food, drug & fragrance controlled release materials
Publications: 10 Patents, 40 Papers
Experience: Ma Xia, is professor, advisor of graduates. Her Ph.D was obtained from Tianjin University of Science & Technology in 2003. Post-doctor was finished in College of Chemistry and Bioengineering, Donghua University in 2008. Her research area focuses on new biologic material, fermentation technology, functional food, and flavour. She has taken charge in several scientific projects. About 30 papers were published. 4 natural invention patents were applied. 2 third progress prize of Shandong Science and Technology and 2 second progress prize of Shandong Science and Technology were gained.

Zhang MianMian, born in August 5, 1988, Henan, China

Current position: A graduate student in Shanghai institute of technology

Chen ShiWen, born in January 28, 1971, Shandong, China

Current position: Doctor of Neurosurgery, associate professor and master's supervisor in Shanghai JiaoTong University Affiliated Sixth People's Hospital
University studies: Clinical medicine in The Second Military Medical University
Scientific interest: Biologic material being used on the medical aspect, Brain injury and Cerebrovascular disease
Publications: 40 Papers
Experience: Chen ShiWen, is an associate professor. His M.D was obtained from The Second Military Medical University in 2005. Post-doctor was finished in Medical College of Shanghai JiaoTong University in 2011. His research area focuses on biologic material being used on the medical aspect, brain injury and cerebrovascular disease. He has taken part in several scientific projects. More than 40 papers were published.