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Research article

Preparation and Release Behavior of Pectin Nanoparticles Loading Doxorubicin

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ABSTRACT: Doxorubicin (DOX) is a highly efficient and broad-spectrum antitumor drug in the treatment of solid tumors. However, DOX can lead to a wide range of biochemical effects on human including inevitable toxicity to the normal tissues which has limited its clinical application. Nanoparticles, as a new kind of vehicle for antitumor drug delivery, can greatly enhance the theraputic effect and reduce the drug toxicity. In this work, we chose pectin (PEC) as carrier material to prepare DOX loading nanoparticles. The blank PEC nanoparticles (PEC-NPs) were prepared by emulsification-ionotropic gelation method and the formulation was optimized by central composite design and response surface method. The drug was loaded in PEC-NPs to obtain DOX loading pectin nanoparticles (DOX-PEC-NPs) by adsorption. The drug loading capacity of PEC-NPs was chiefly affected by ratio of DOX to PEC. The time, pH value and temperature of adsorption showed some influence on drug loading. Both PEC-NPs and DOX-PEC-NPs were sphere-like in shape with a mean diameter of (266.93 ± 1.70) nm and (287.70 ± 1.21) nm, respectively. The entrapment efficiency and drug loading rate of DOX-PEC-NPs was (91.71 ± 0.50)% and (17.17 ± 0.24) %, respectively. Compared with DOX, DOX-PEC-NPs showed apparent sustained release behavior with a burst release, which followed the double exponential diphase kinetics equation well.

KEYWORDS: Doxorubicin; Pectin; Nanoparticles; Drug loading; Drug release.

INTRODUCTION

The incidence of cancer is rising rapidly in recent decades, which has become a serious threat to human life and health. Most patients have to accept chemotherapy instead of the surgical treatment because the tumor has developed into terminal stage when it is diagnosed[1-2]. However, the clinical efficacy of chemotherapy is seriously hindered by the subsequent side effects[3], which has caused increasing attention among experts around the world recently. Nano drug delivery system is a new kind of microparticle sustained release system in nanometer size, which can wrap chemotherapy drugs into carrier materials[4]. The microparticle drug delivery system can reduce the side effects on normal tissues and increase the concentration of the drugs in tumor by sustained release and targeting action. Besides, they are characterized with the advantages of tunable pharmacokinetic properties, larger surface area, improved drug solubility, extended drug half-life and the flexibility of controlled release[5]. Depending on the properties of drug and carrier materials, nanoparticles can be prepared by

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various methods such as microemulsion method, desolvation method, ion induction-chemical crosslinking method, solvent diffusion method, multiple emulsion method and so on[6-10].

Doxorubicin is a commonly used clinical chemotherapy drug. Direct application of DOX leads to great damages to normal cells due to its obvious heart toxicity and inhibitory effect on spinal marrows[11-12]. Nanoparticles, as an ideal carrier for DOX, can reduce the toxicity and improve the drug release behavior and targeting action[13-14]. Pectin is a natural nontoxic and extensively existed polysaccharide with well biocompatibility, bioadhesiveness and biodegradability[15-17]. It was a widely used carrier material in sustained and controlled release preparations as well as in microparticle drug delivery systems. It can serve as carrier for gels, films, microspheres, nanoparticles and other kinds of drug delivery systems[18-21]. According to the previous researches, modified citrus pectin demonstrated particular anti-tumor effects by the means of selective inhibition to the growth, metastasis and angiogenesis of cancer cells[22].

In this work, we chose pectin as carrier material to prepare DOX loading nanoparticles in order to improve the toxicity and efficacy of DOX. The formulation of blank pectin nanoparticles (PEC-NPs) was optimized and the drug loading capacity was investigated. The DOX loading PEC-NPs (DOX-PEC-NPs) were characterized and the drug release behavior was studied.

MATERIALS AND METHODS

MATERIALS

Doxorubicin (DOX, Lot M1101104) was provided by Haizheng pharmaceutical industry of Zhejiang Province, China. Pectin (PEC, Lot SK14191) was provided by CPKelco Company, Danmark. Oleic acid and Sodium bis(2-ethylhexyl) sulfosuccinate (AOT) was purchased from Aladdin company, Shanghai, China. All other chemicals used were of analytical reagent grade.

PREPARATION OF PEC-NPS AND DOX-PEC-NPS

PEC-NPs were prepared with reference to reported method[6,23]. Briefly, AOT was dissolved in oleic acid and pectin solution was added into it slowly under stirring. The coarse emulsion was ultrasonic treated to form a clear transparent microemulsion. The propyl alcohol solution of calcium chloride was dispersed in oleic acid well and mixed with the microemulsion for gelation of pectin. The mixture liquid was then centrifuged to separate PEC-NPs. The precipitate was washed with 50% and anhydrous ethanol followed by centrifugation and then dried by vacuum to get white PEC-NPs powders.

DOX-PEC-NPs were prepared by dispersing PEC-NPs in DOX solution for drug adsorption.

FORMULATION OPTIMIZATION OF PEC-NPS BY RSM

According to the results of single factor tests, central composite design (CCD) and response surface method (RSM) was adopted to optimize the formulation of PEC-NPs. Four factors were investigated, including the oil/water phase volume ratio (A, v/v), the concentration of PEC (B, %, w/v), the amount of AOT (C, g) and the concentration of calcium chloride dihydrate in propyl alcohol solution (D, %, w/v). Particle size (Y_1) and particle size distribution index (PDI, Y2) was used for evaluation. The tests were arranged by central composite design. The level in code and actual value of each factor and the experimental scheme was listed in Table 1 and Table 2, respectively. The data were processed for both response surface diagram plotting and mathematic model fitting by using Design-Expert 8.0 software. The optimal model was used to predict optimized formulation and validation test was done for three times in parallel.

Table 1 Factors and levels in code and actual value

Levels in code of each factors	-2	-1	0	1	2
A (oil/water phase volume ratio)	40:1	32.5:1	25:1	17.5:1	10:1
B (concentration of PEC/%)	1.0	1.75	2.50	3.25	4.0
C (amount of AOT/g)	0.05	0.0875	0.125	0.1625	0.2
D (concentration of calcium chloride dihydrate in propyl alcohol/%)	1.0	1.75	2.50	3.25	4.0

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DRUG LOADING CAPACITY OF PEC-NPS

DOX-PEC-NPs were prepared by using the optimal formulation obtained from 2.3. The drug loading capacity of PEC-NPs was investigated under different drug-carrier ratio, adsorption time, adsorption temperature and pH.

The entrapment efficiency (EE%) and drug loading rate (DL%) of DOX-PEC-NPs was measured by UV-Vis spectrophotometric method at wavelength of 480 nm. The suspension of PEC-NPs and DOX-PEC-NPs were centrifuged, respectively. The supernatant of PEC-NPs was used as blank reference, and the absorbance of DOX-PEC-NPs' supernatant was measured to calculate EE%. Similarly, the precipitate was dissolved in 0.1 mol/L sodium citrate solution and the absorbance was measured to calculate DL%. The formula to calculate EE% and DL% was as follows.

W_T, W_f, and W_N represent the total weight of DOX used, the weight of free DOX unentrapped and the weight of DOX-PEC-NPs for test, respectively.

$$EE\% = \frac{W_T - W_f}{W_T} \times 100\% \qquad DL\% = \frac{W_T - W_f}{W_N} \times 100\%$$

CHARACTERIZATION OF PEC-NPS AND DOX-PEC-NPS

The particle size and zeta potential of PEC-NPs and DOX-PEC-NPs was measured by using Nanosizer (Nano-zs+MPT-2, Melvern Instruments Ltd.). The morphology was observed by using transmission electronic microscope (TECNAI 10, Dutch PHILIPS Company), scanning electronic microscope (XL-30ESEM, Dutch PHILIPS Company) and atomic force microscope (Multimode Nanoscope-V, Veeco Percision Instrument Company of USA).

RELEASE BEHAVIORS OF DOX-PEC-NPS In Vitro

Dialysis method was used to investigate the drug release behavior of DOX-PEC-NPs. An appropriate volume of DOX-PEC-NPs suspension was placed in a dialysis bag and then sealed. The dialysis bag was placed in a sample bottle containing release medium and shaken at 100 r/min in an air bath oscillator at 37.0 ± 0.5 °C. Samples were taken at 0.5、1.0、1.5、2.0、4.0、8.0、12.0、24.0、36.0、48.0 h. The concentration of release solution was measured by UV-Vis spectrophotometric method at wavelength of 480 nm. The accumulative release of drug was calculated as percentage and plotted versus time. The data was processed for drug release model fitting.

STATISTICAL ANALYSIS

Data were expressed as mean ± standard deviation ($\bar{x} \pm SD$), and one-way ANOVA test was used for statistical analysis with SPSS19.0 software. For a value of $p < 0.05$, the difference was considered statistically significant.

RESULTS AND DISCUSSIONS

FORMULATION OPTIMIZATION OF PEC-NPS

PEC-NPs were prepared according to the experiment schemes arranged by central composite design and the results of particle size and PDI were listed in Table 2. The relationship between particle size (Y_1) or PDI (Y_2) and the four factors (A, B, C and D) was analyzed by plotting both three-dimensional response surface diagrams and two-dimensional contour diagrams (Figure 1 and Figure 2). All of the four factors, oil/water phase volume ratio (A), concentration of PEC (B), amount of AOT (C) and concentration of calcium chloride dihydrate in propyl alcohol (D), affected both particle size and PDI of PEC-NPs significantly with similar trend. As shown in Figure 1 and 2, both particle size and PDI of PEC-NPs turned bigger with the increase of the level of B, C and D, while became smaller first and then bigger with the increase of oil/water phase volume ratio.

Table 2 Experimental scheme and results of central composite design

No.	A	B	C	D	Y_1	Y_2
1	-1	-1	-1	-1	376.73	0.24
2	1	-1	-1	-1	311.43	0.16
3	-1	1	-1	-1	703.70	0.64
4	1	1	-1	-1	329.83	0.25
5	-1	-1	1	-1	444.43	0.31
6	1	-1	1	-1	1087.10	0.86
7	-1	1	1	-1	617.60	0.52

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8	1	1	1	-1	668.00	0.56
9	-1	-1	-1	1	434.73	0.37
10	1	-1	-1	1	299.07	0.19
11	-1	1	-1	1	886.13	0.74
12	1	1	-1	1	404.77	0.37
13	-1	-1	1	1	442.03	0.35
14	1	-1	1	1	897.35	0.70
15	-1	1	1	1	525.07	0.50
16	1	1	1	0	725.70	0.59
17	-2	0	0	0	1273.67	0.86
18	2	0	0	0	1033.97	0.68
19	0	-2	0	0	296.63	0.20
20	0	2	0	0	480.93	0.43
21	0	0	-2	0	324.63	0.20
22	0	0	2	0	563.43	0.42
23	0	0	0	-2	330.65	0.17
24	0	0	0	2	300.27	0.36
25 ~ 30	0	0	0	0	358.98 ± 34.68	0.25 ± 0.03

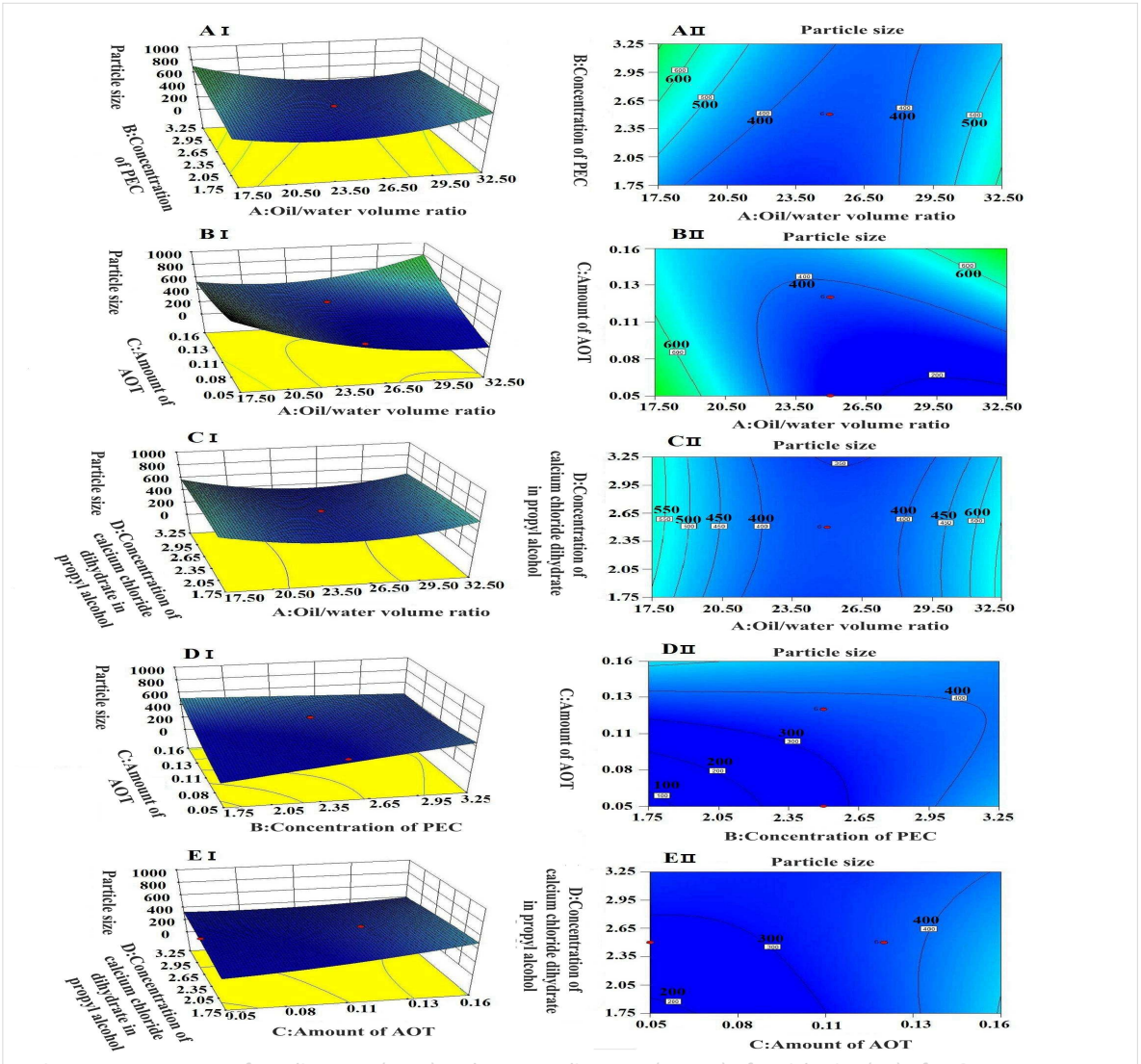

Figure 1. Response surface diagrams (AI-EI) and contour diagrams (AII- EII) of paticle size (Y1) of PEC-NPs.

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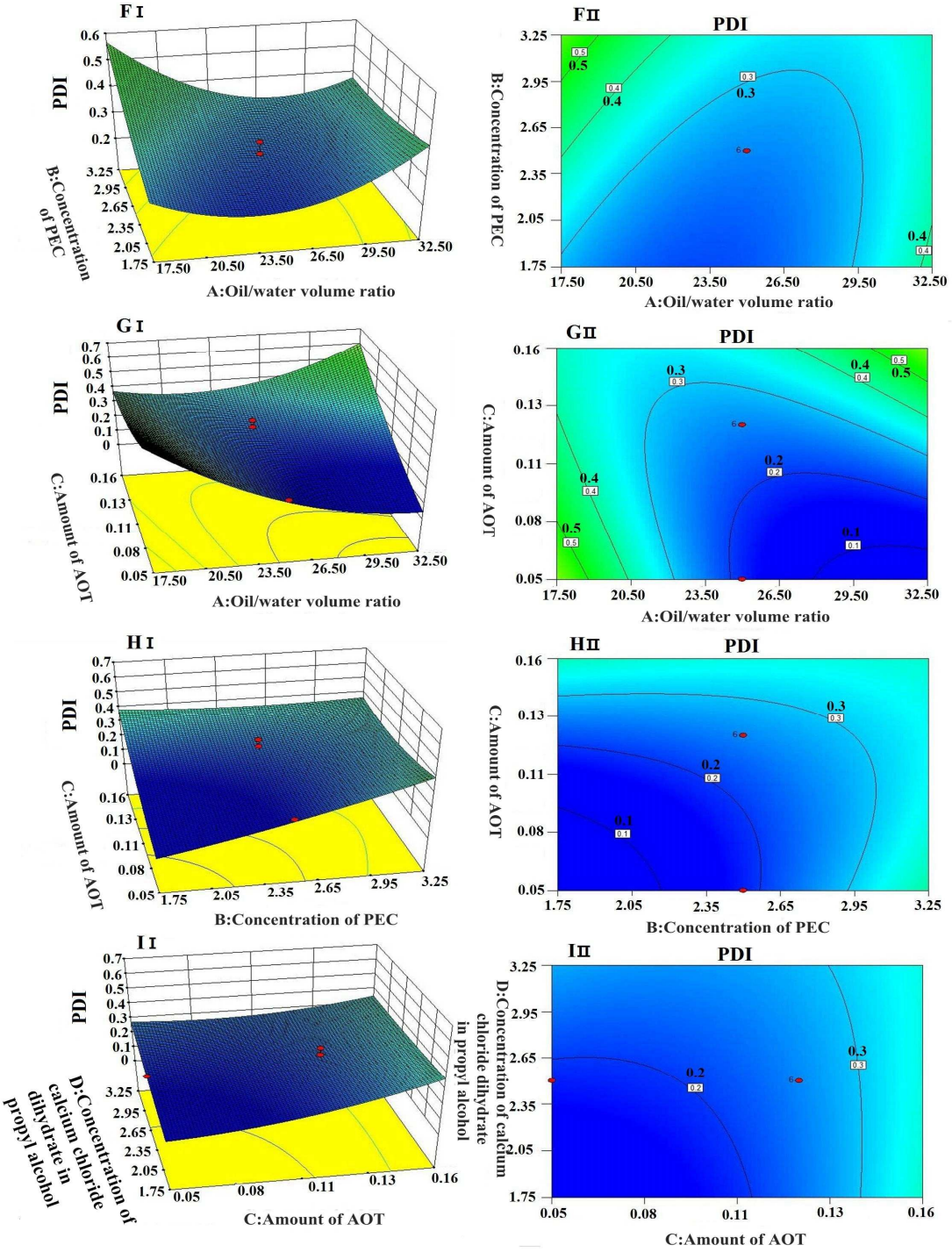

Figure 2. Response surface diagrams (FI- I-I) and contour diagrams (FII – I-II) of the PDI (Y2) of PEC-NPs

Meanwhile, the data were fitted by both multiple linear regression model and quadratic polynomial model. The quadratic polynomial model fitted the data better, which showed a high goodness of fitting (r) and a low P value of statistical significance. The optimal quadratic polynomial equations were as follows for Y_1 and Y_2 both with $p < 0.0001$. The r value for Y_1 and Y_2 was 0.9681 and 0.9552, respectively.

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$$Y_1 = 368.3 - 11.45A + 35.35B + 85.43C - 5.98D - 99.34AB + 144.82AC - 23.37AD - 82.82BC - 43.05 CD + 196.05A^2 + 18.61C^2 - 15.75D^2$$

$$Y_2 = 0.26 - 0.014A + 0.060B + 0.079C + 0.024D - 0.080AB + 0.13AC - 0.068BC - 0.033CD + 0.14A^2 + 0.022B^2 + 0.020C^2 + 0.007D^2$$

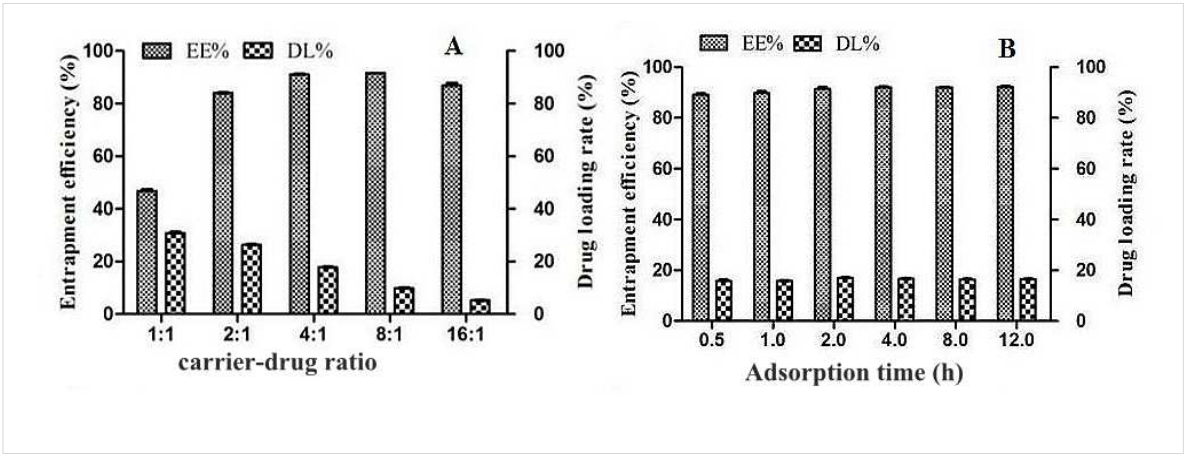
The optimized formulation of PEC-NPs was predicted by the models. The oil/water phase volume ratio was 29.42:1, the concentration of PEC 2.75%, the amount of AOT was 0.07 g and the concentration of calcium chloride dihydrate in propyl alcohol was 2.93%. Three batches of PEC-NPs were prepared according to the predicted formulation for validation test. The results showed that the deviation between the observed and predicted value of particle size and PDI were all less than 5% (Table 3), indicating the good predictability and practicability of the mathematical model we obtained.

Table 3. The results of validation tests

n	Particle size（nm）			PDI		
	Predicted	Observed	Deviation (%)	Predicted	Observed	Deviation (%)
1	267.38	265.30	0.78	0.199	0.190	4.52
2	267.38	264.90	0.93	0.199	0.202	-1.51
3	267.38	266.80	0.22	0.199	0.205	-3.02

DRUG LOADING CAPACITY OF DOX-PEC-NPS

Results of single factor tests for drug loading capacity were showed in Figure 3. The carrier-drug ratio, adsorption time, adsorption temperature and adsorption pH had influence on the EE% and DL% of DOX-PEC-NPs to different extent with significant difference among levels ($p<0.05$). The increase of carrier-drug ratio enhanced the EE% but lowered down DL%. Adsorption of drug became stable with time prolonged. When temperature was higher than 20 °C, the EE% was more than 90% and DL% was around 17%. The EE% and DL% showed a slight raise with the increase of pH value of adsorption solution. The EE% and DL% of DOX-PEC-NPs was finally optimized to (91.71 ± 0.50)% and (17.17 ± 0.24)%, respectively. PEC is a kind of negative charged polysaccharide, which may cause electrostatic interaction with positive charged DOX. This resulted in the high EE% and DL% although adsorption method was used for drug loading.



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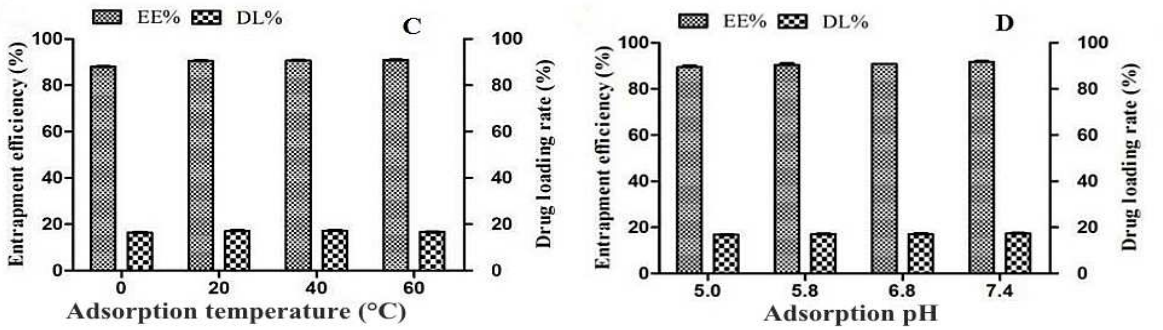

Figure 3 Influence of the carrier-drug ratio (A), adsorption time (B), adsorption temperature (C) and adsorption pH on EE% and DL% of DOX-PEC-NPs: (`x ± s, n=3).

CHARACTERIZATION OF PEC-NPS AND DOX-PEC-NPS

The morphology of PEC-NPs and DOX-PEC-NPs was observed by transmission electron microscope (TEM), scanning electron microscope (SEM) and atomic force microscope (AFM) (Figure 4). Both PEC-NPs and DOX-PEG-NPs were tiny spherical particles with narrow size distribution. The particle size of PEC-NPs was (266.93 ± 1.70) nm with a PDI of (0.20 ± 0.01), and the zeta potential was (-22.10 ± 0.53) mV. After loading DOX, the particle size increased a little to (287.70 ± 1.21) nm with a PDI of (0.15 ± 0.02), and the zeta potential changed slightly to (-20.40 ± 1.50).

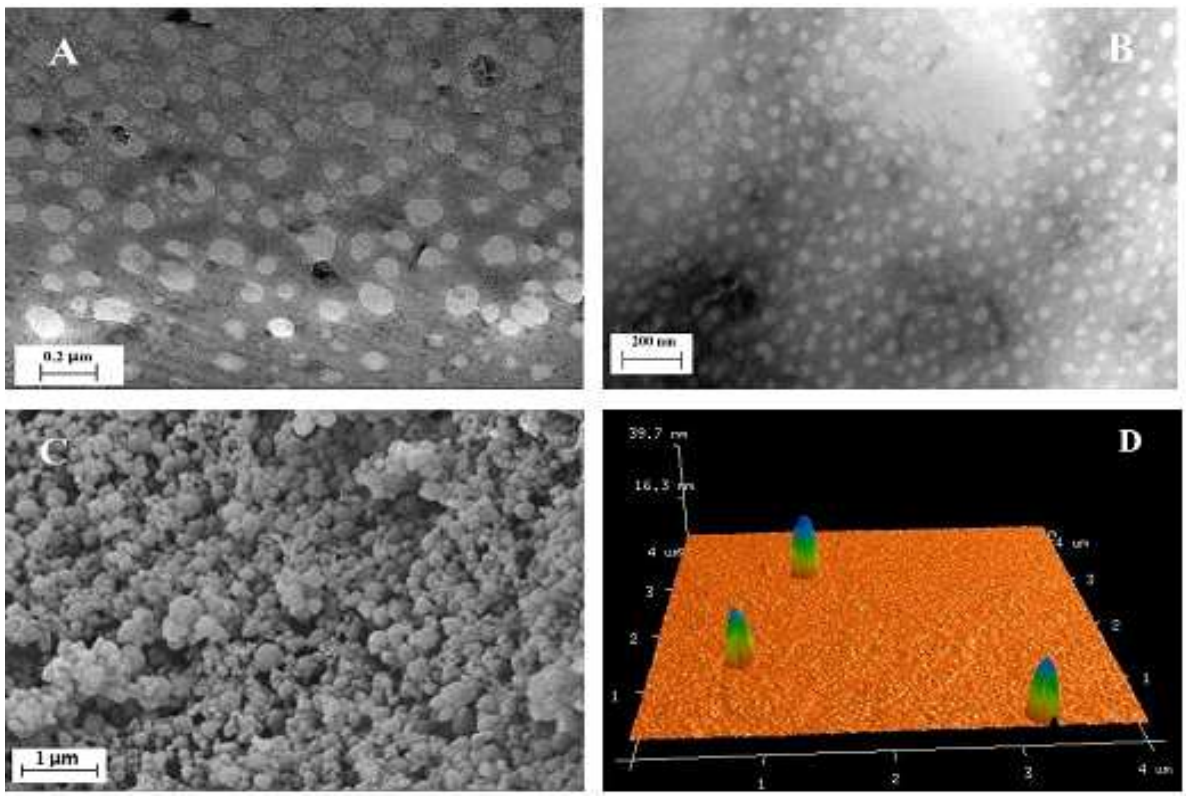

Figure 4. TEM micrographs of DOX-PEC-NPs (A) and PEC-NPs (B), SEM micrograph of DOX-PEC-NPs (C) and AFM micrograph of PEC-NPs (D).

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RELEASE BEHAVIORS OF DOX-PEC-NPS IN DIFFERENT MEDIUM

The accumulative release curves of DOX-PEC-NPs in phosphate buffer saline (PBS, pH=6.8) and normal saline were illustrated in Figure 5. The release behaviors of DOX-PEC-NPs in PBS and normal saline were basically the same ($P > 0.05$) and both of them were significantly slower than DOX ($P < 0.05$). DOX-PEC-NPs produced a typical sustained release with a slightly fast burst release, which was about 20% at 0.5 h. A mild slow release was observed after 4 h, and around 80% of DOX was released after 24 h.

The model fitting of drug release data was listed in Table 4 by using zero order kinetics, first order kinetics, Higuchi and double exponential diphase kinetics equation. The results showed that the value of goodness of fitting (r) was almost 1 for double exponential diphase kinetics equation. It indicated that the model explained the release behaviors of DOX-PEC-NPs in different medium well.

In this work, DOX was loaded by adsorption so that quite a part of drug was just existed on the surface of nanoparticles, which was apt to dissolve easily in the medium to cause the first-phase quick release at the initial stage. The rest part of drug might entered the matrix of PEC-NPs and had somewhat strong interaction with PEC, thus generated the second-phase mild slow release after 4 h.

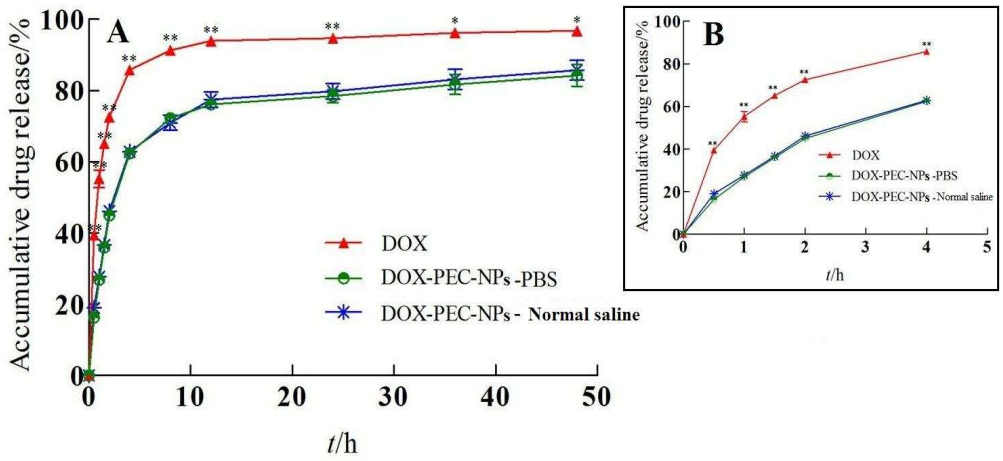

Figure 5. Drug release behavior of DOX and DOX-PEC-NPs (A: 0-48 h, B: 0-4 h, *p < 0.05, **p < 0.01).

Table 4. Model fitting of drug release data of DOX-PEC-NPs.

Medium	Models	Equations	r
PBS	Zero order kinetics	Q = 0.362 + 0.013t	0.548
	First order kinetics	ln(1-Q) = -0.513 – 0.034t	0.722
	Higuchi equation	$Q = 0.215 + 0.113t^{1/2}$	0.773
	Two-phase kinetics	$Q = 1.320 – (0.595e^{-0.005t} + 0.722e^{-0.461t})$	1.000
Normal saline	Zero order kinetics	Q = 0.369 + 0.013t	0.560
	First order kinetics	ln(1-Q) = - 0.520 – 0.036t	0.750
	Higuchi equation	$Q = 0.222 + 0.114t^{1/2}$	0.785
	Two-phase kinetics	$Q = 0.894 – (0.206e^{-0.035t} + 0.678e^{-0.505t})$	0.998

CONCLUSION

PEC-NPs were prepared successfully by emulsification-ionotropic gelation method, and DOX-PEC-NPs of high drug loading rate were attained by drug adsorption method. DOX-PEC-NPs were a kind of nano-size sphere like microparticle drug delivery system with good dispersity, which showed a typical diphase sustained release of DOX. The high drug loading capacity and sustained release behavior allowed PEC-NPs to be an ideal drug delivery system for DOX, and even for other chemotherapy drugs as well. Moreover, the anti-tumor activity and

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pharmacokinetics behavior of DOX-PEC-NPs should be further investigated to verify its advantages.

REFERENCES

1.Gong LS, Zhang YD, Zhou SB. The Biodistribution of magnetic albumin nanoparticles containing doxorubicin in rats[J]. China Journal of Modern Medicine. 2001;11(3):8-10.
2.Menon SK, Mistry BR, Joshi KV, et a1. Analytical detection and method development of anticancer drug Gemcitabine HCl using gold nanoparticles[J]. Spectrochim Acta A Mol Biomol Spectrosc. 2012; 10(94A):235-242.
3.Stein W, Bates S, Fojo T. Intractable cancers: the many faces of multidrug resistance and the many targets it presents for therapeutic attack. Current Drug Targets. 2004;5(4):333-346.
4.Kingsley JD, Dou H, Morehead J, et al. Nanotechnology: a focus on nanoparticles as a drug delivery system[J]. Journal of Neuroimmune Pharmacology. 2006;1(3):340-350.
5.Joseph MM, Aravind SR, George SK, et al. Anticancer activity of galactoxyloglucan polysaccharide-conjugated doxorubicin nanoparticles: Mechanistic insights and interactome analysis. Eur J Pharm Biopharm. 2015; 93:183-195.
6.You JO. Efficient gene delivery with calcium-alginate nanoparticles[D]. University of Southern California. 2006.
7.Mao SJ, Hou SX, Zhang LK. Preparation and liver targeting properties of glycyrrhizic acid surface modified Valaciclovir albumin nanoparticles[J]. Journal of Biomedical Engineering. 2004;21(4):570-574.
8.Chen J, Hu Q, Hou ZQ, et al. Chitosan nanoparticles prepared by ion induction combined with chemical crosslinking method and preliminary studies about its being a targeting control-released carrier for drugs[J]. Chinese Pharmaceutical Journal. 2010; (18): 1400-1403.
9.Xu H, Hu FQ, Ying XY, et al. Preparation of evaluation of in vitro and in vivo of alginate nanoparticles loading insulin[J]. Chinese Pharmaceutical Journal. 2006; 41(6): 434-437.
10.Zhou XY, Shen Q, Li ML, et al. Preparation of Saussurea nanoparticles by multiple emulsion method[J]. Lishizhen Medicine and Materia Medica Research. 2010; 21(7):1726-1727.
11.Vanderwaal RP, Griffith CL, Wright WD. Delaying S-phase progression rescues cells from heat-induced S-phase hypertoxicity[J]. Journal of Cellular Physiology. 2001;14(2):81-82.
12.Taheri A, Dinarvand R, Atyabi F, et al. Targeted delivery of methotrexate to tumor cells using biotin functionalized methotrexate human serum albumin conjugated nanoparticles[J]. J Biomed Nanotechnol. 2011;7(6):743-753.
13.Widder KJ, Marino PA, Morris RM. Selective targeting of magnetic albumn microspheres to the yoshida Sarcoma: Ultrastructural evaluation of microsphere disposition[J]. European Journal of Cancer & Clinical Oncology. 1983;(1):96-98.
14.Brusentsov NA, Gogosov VV, Brusentsova TN. Evaluation of ferromagnetic fluids and suspensions for the site-specific radiofrequency-induced hyperthermia of MX11 sarcoma cells in vitro[J]. Journal of Magnetism and Magnetic Materials. 2001;11(2):934-940.
15.Ashford M, Fell J, Attwood D. Enhanced pectin degradation in the presence of calcium-implications for colonic delivery[J]. Proc Int Symp Controlled Release Bioact Mater. 1994; 21:750-751.
16.Sriamornsak P. Chemistry of pectin and its pharmaceutical uses: A review[J]. Silpakorn University International Journal. 2003;3(1-2):206-228.
17.Mishra R, Banthia A, Majeed A. Pectin based formulations for biomedical applications: A review[J]. Asian Journal of Pharmaceutical & Clinical Research. 2012;5(4):1-7.
18.Liu L, Fishman ML, Kost J, et al. Pectin-based systems for colon-specific drug delivery via oral route[J]. Biomaterials. 2003;24(19):3333-3343.
19.Sriamornsak P. Application of pectin in oral drug delivery[J]. Expert opinion on drug delivery. 2011; 8(8):1009-1023.
20.Yoshimura T, Sengoku K, Fujioka R. Pectin-based surperabsorbent hydrogels crosslinked by some chemicals: Synthesis and characterization[J]. Polymer bulletin. 2005; 55(1-2):123-129.
21.Cheng K, Lim L-Y. Insulin-loaded calcium pectinate nanoparticles: Effects of pectin molecular weight and formulation pH[J]. Drug development and industrial pharmacy. 2004;30(4):359-367.
22.Glinsky VV, Raz A. Modified citrus pectin anti-metastatic properties: One bullet, multiple targets[J]. Carbohydrate research. 2009; 344(14):1788-1791.
23.Li S, Wang XT, Zhang XB, et al. Studies on alginate-chitosan microcapsules and renal arterial embolization in rabbits[J]. Journal of Controlled Releaase. 2002;84(3):87-98.

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