PREPARATION OF VINORELBINE TARTRATE THERMOSENSITIVE LIPOSOMES AND MEASUREMENT OF ITS ENCAPSULATION EFFICIENCY

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ABSTRACT

Objective*: This paper aims to explore the preparation of vinorelbine tartrate (VRT) thermosensitive liposomes and determine its encapsulation efficiency.*

Methods*: Preparation of VRT thermosensitive liposomes was carried out by pH gradient method. The VRT content in liposomes was determined by high performance liquid chromatograph (HPLC). Separation of liposomes and free drugs was done with microcolumn centrifugation method. The influence on type of dextrangel and elution solvent as well as eluted volume of vinorelbine tartrate and free drug was closely observed.*

Results*: The average particle size and average content of vinorelbine tartrate were (94.8 ± 1.5) nm, (1.84±0.04) mg/mL respectively. The dextrangel G-25 was used for preparation of microcolumn, in which 4 cm was the best column height. The elution solvent used was PBS buffer and gradient elution volume method was employed in separation of liposomes and free drugs. The encapsulation efficiency of three batches vinorelbine tartrate were recorded as (95.76 ± 1.43)%, (91.13 ± 1.24)% and (94.13 ± 2.05)%, with respective elution recovery rate at (98.5 ± 2.41)%, (93.6 ± 1.85)% and 96.8 ± 1.67)%.*

Conclusion*: Preparation of vinorelbine tartrate is relatively a stable process technology with large drug loading, which resulted in high encapsulation efficiency. Moreover, there are accurate and convenient methods for measurements of content and encapsulation efficiency.*

Keywords*: vinorelbine tartrate; thermosensitive liposomes; preparation and encapsulation efficiency.*

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Introduction

Vinorelbine is a semi-synthetic vinblastine. In 1989, French Pierre Fabre pharmaceutical company first produced and listed vinorelbine tartrate for injection, that is, VRT. Vinorelbine tartrate has important practical applications in anti-tumor activity, especially in treatment of non-small cell lung cancer and advanced breast cancer. With good effect, it has become the world's most commonly used antineoplastic chemotherapy drug in the medical field[1].

Vinorelbine tartrate (Figure 1) has dose-dependent toxicities in nerve, respiratory and gastrointestinal tract and so on. In view of this, most researchers began to pay attention to study on targeting drug delivery system such as liposomes and microspheres, in order to minimize the resulting toxic and side effects, and thus significantly enhance clinical use compliance[2-4]. In this course of study, dipalmitoylphosphatidylcholine, stearyl phosphatidylglycerol, hydroxylated acylglycerol phosphatidylcholine mixed phospholipids were used as prescriptions of blank liposomes, that is,

DPPC (Figure 2), DSPG (Figure 3), MSPC (Figure 4). Vinorelbine tartrate was loaded into the blank liposomes via pH gradient method to make vinorelbine tartrate thermosensitive liposomes.

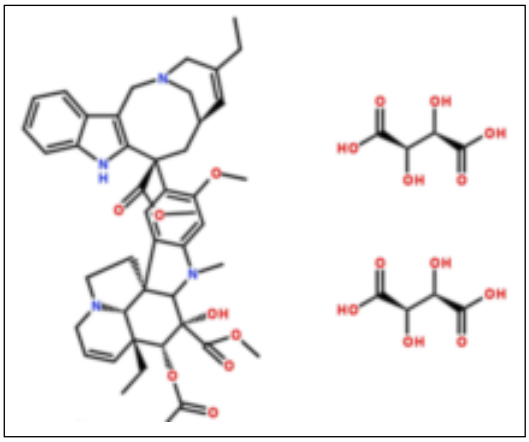

Figure 1: Molecular formula of vinorelbine tartrate.

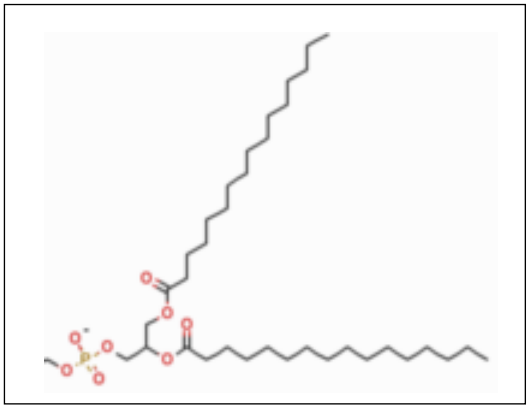

Figure 2: DPPC structural formula.

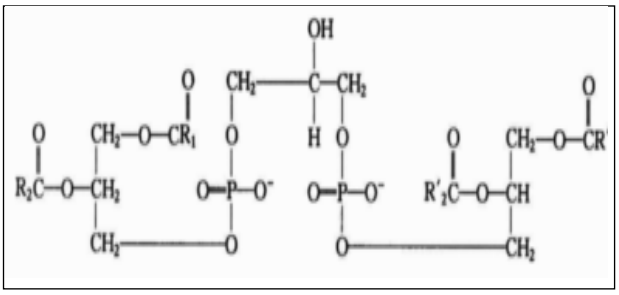

Figure 3: DSPG structural formula.

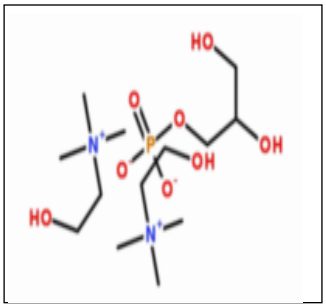

Figure 4: MSPC structural formula.

In this research work, the separation of VRT thermosensitive liposomes was done by microcolumn centrifugation and factors influencing the separation were explored. In addition, method determination for encapsulation efficiency was also established. The detailed report is as follows.

General information

Equipment used include HITACHI high performance liquid chromatograph (HPLC), EZChro Elite ClientL-2000 chemical workstation; Scientz-II D ultrasonic cell grinder; TDL-4 desktop centrifuge; Nanophox laser particle size analyzer; vinorelbine tartrate produced by Hangzhou Minsheng Pharma. Co., Ltd., DPPC, DSPG, MSPC manufactured by Northem Lipids, Genzyme, Genzyme, USA; 2 mL disposable plastic syringe; 1.3 cm x 10.0 cm plastic centrifuge tube; dextrangel G-25; chromatographically pure methanol, distilled water, plus analytically pure remaining reagents.

Methods and results

Preparation of liposomes

The first step was blank liposomes preparation. Weigh appropriate amount of DPPC-DSPG-MSPC mixed phospholipids and dissolved using chloroform - methanol mixture. Subsequently, vacuum rotary evaporation was utilized to remove organic solvent and to build a uniform film in eggplant bottle wall; add sodium citrate buffer for rotating hydration, followed by probe ultrasound to obtain blue opalescence translucent liquid, i.e. liposomal solution; select appropriate amount of liposomal solution for the top of dextran G-50 gel column, followed by elution with PBS buffer and collection of liposome, to generate a pH gradient in liposomes and external water phase[5]. Secondly, prepare VRT liposomes. Weigh appropriate amount of blank liposomes and VRT solution for even mixture, followed by static bath incubation for 1 h at 30 -35°C to obtain VRT thermosensitive liposomes. The prepared liposomes were in colloidal solutions with blue opalescence and stored in refrigerator at 4°C.

Determination of liposome particle size and content

The particle size of liposomes was measured by utilizing laser dynamic diffraction method. About 0.1 mL of VRT thermosensitive liposomal suspension was diluted to 5 mL with distilled water.

During the measurement, intensity of the laser light source was adjusted to about 55%[6]. Three measurement was obtained with the average liposome size of (94.8 ± 1.5) nm , as shown in Figure 5.

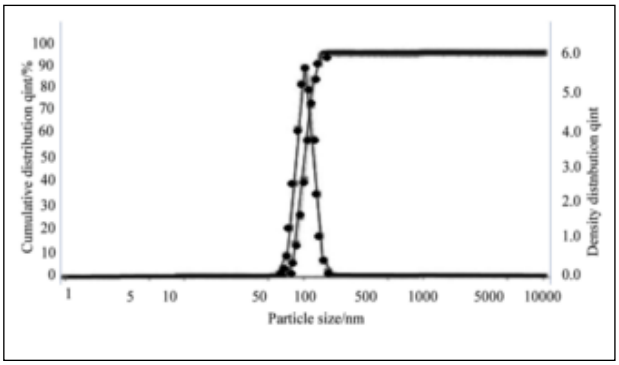

Figure 5: Particle size distributions of VRT liposomes.

Following test was conducted to establish the content d for VRT thermosensitive liposomes. The chromatographic condition was as follows: Venusil Akasil C18 column was utilized, mobile phase used was decanesulfonic acid sodium methanol solution -0.05 mol/L sodium dihydrogen phosphate, the flow rate was set at 1.0 mL/min, the column temperature was 40°C[7] and the detected wavelength was 267 nm. During the content determination, 0.3 mL VRT thermosensitive liposomal suspension was added to the flask (10 mL), and subjected to methanol with membrane Intraprep Permeabilization reagent flow and constant volume process before obtaining test sample. At the same time, mobile phase was used to prepare VRT reference solution. The test sample and reference solution were measured with high performance liquid chromatograph while VRT content in liposomes was determined by using external standard method. Accordingly, the average content of VRT in liposomes obtained was (1.84 ± 0.04) mg/mL .

Finally, the established method validation was conducted. Based on the above chromatogram conditions, thorough investigation and analysis of indicators such as specificity, linear range and sample recovery rate, precision, stability and repeatability was performed[8].

Determination of encapsulation efficiency

In this section, method for determining encapsulation efficiency was established. The drug-loaded liposomes were first separated from free drug by microcolumn centrifugation. The bottom pad filter of plastic syringe was removed and 4 mL of distilled water-treated dextrangel was added using the syringe. After that, excess water was removed by centrifugal. The upper sample of double diluted VRT was taken, followed by centrifugation at 1500 rmin-1 for 2 minutes. The centrifugation process was repeated after adding 100 μL distilled water. This was followed by elution into several tubes[9-13]. The results showed that liposomes could be easily eluted. The first four tubes were eluted completely, so the elution conditions of liposomes were as follows: 1500 r min-1 centrifugation for 2 minutes. The first tube was directly eluted after loading and each of 2-4 tubes had 100 μL of elution volume.

The next stage observation on dextrangel type and elution solvent was performed. Dextrangels, which are often used to separate liposomes from free drugs, are subjected to encapsulation efficiency determination. The microcolumn was prepared by dextrangel G-50 and G-25 respectively. The column was fixed at 4 cm with 0.1 mL of VRT aqueous solution added to the microcolumn, followed by elution with distilled water and PBS buffer as the elution solvent[14]. The results demonstrated that choice of dextrangel and elution solvent of different models had a significant effect on elution of free drug, as shown in Figure 6 . It could be noted that dextrangel G-25 and acid-base elution method could promote the separation of liposomes from VRT free drugs.

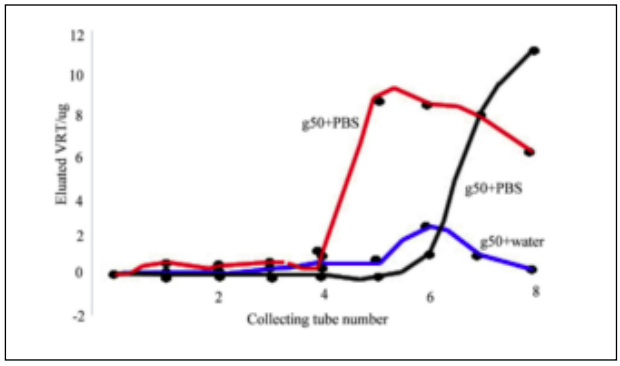

Figure 6: Impact of dextrangel model, elution solvent.

Subsequent study on column height was conducted. Dextrangel G-25 was loaded into the column with addition of 0.1 mL VRT aqueous solution into the microcolumn. This was followed by acid-base elution and collection of eluent onto the first 4 tubes. The amount of elution drug per tube is presented in Table 1. The results showed that free drug in the column was unable to be separated from liposome due to premature elution when the column height was less than or equal to 3.5 cm.

Consequently, separation of liposome from free drug could be achieved with the column height of 4 cm.

Column height (cm)	Tube 1	Tube 2	Tube 3	Tube 4
3	0	0.36±0.08	0.62±0.17	0.30±0.12
3.5	0	0.04±0.02	0.22±0.03	0
4	0	0	0	0

Table 1: Effect of column height (± s).

Next, investigation on the elution volume was studied. An amount of 0.1 mL of VRT aqueous solution (0.4mg/mL) was added to 4 dextrangel G-25 microcolumns. For the initial 2-4 tubes, 100 μL PBSI was taken as elution solvent while for the next 5-10 tubes, PBS2 was used as elution drug solution. The eluent from tube 5-10 was transferred into 2 mL volumetric flask, followed by constant volume process and determination. The elution recovery is displayed in Table 2, and D program has the highest elution efficiency.

Tube group (μL)							Elution recovery (%)
Program	5	6	7	8	9	10	
A	300	300	300	400	400	400	62.3±8.5
B	200	300	200	300	400	400	65.6±9.6
C	300	300	400	500	400	300	73.9±12.3
D	300	700	500	300	100	100	92.2±13.5

Table 2: Effect of elution volume (± s).

Finally, the encapsulation efficiency was determined. Based on optimal elution conditions, detection method of encapsulation efficiency of VRT liposomes was decided. The elution separation curve was drawn to calculate blank and the average recovery of microcolumn. Encapsulation efficiency and recovery rate of three batches of VRT liposomes were measured. The results (as shown in Table 3) demonstrated stable preparation process and encapsulation efficiency of VRT liposomes. At the same time, measurement of encapsulation efficiency also showed good repeatability.

Batch	EE (%)	Recovery (%)
20160822	95.76±1.43	98.3±2.45
20160825	91.10±1.22	93.5±1.84
20160906	94.12±2.04	96.6±1.64

Table 3: Measurement results of VRT liposome encapsulation efficiency (± s).

Discussions

Vinorelbine is a weak alkaline drug. Its solubility and dissociation that has pH-dependent problems, is suitable for pH gradient method in drug loading. PH gradients affect equilibrium transmembrane distribution of certain weak base and acids. Usually, most chemotherapy drugs are weak bases (15). Experimental data showed that such chemotherapeutic drugs may accumulate in liposomal vesicles depending on pH gradient. In this study, a blank liposome with pH gradient was made using column chromatography, to be mixed and incubated with VRT aqueous solution in order to prepare VRT thermosensitive liposomes. The obtained average particle size and content was (94.8 ± 1.5) nm and (1.84 ± 0.04) mg/mL respectively. Concurrently, the encapsulation efficiency exceeded 90%.

The key to establish encapsulation efficiency assay is on how to achieve sufficient and effective separation of liposomes from free drugs. Studies have shown that gel models, elution solvents, column height and elution volumes exert obvious influence on separation of liposomes and free drugs. For VRT liposomes, G-25 has better elution effect compared to G-50. Besides, choice of elution solvent has a significant impact on separation of liposomes and free drugs as well as elution recovery efficiency of free drugs. By using pH-dependent characteristics of VRT solubility and dissociation, selection of weakly-alkaline PBS for elution of drug-loaded liposomes retained free drugs in gel column and enhanced the separation effect[16]. Dissociation of VRT in the elution solvent was improved by selecting weakly-acid PBS for elution of free drug in the gel column, which enhanced the elution efficiency. In addition, during transfer of free drug with elution solvent, gradient elution volume method improved the elution efficiency of VRT free drug. During elution recovery of VRT free drug, the elution efficiency could be significantly increased by addition of methanol.

In this study, we separated liposome from VRT free drug by dextrangel G25 microcolumn centrifugation to measure VRT liposome encapsulation efficiency. The recovery rate of column drug and recovery of column sample were tested by high, medium and low concentrations of VRT solution. The results demonstrated that this method could effectively separate liposomes and free drugs

in VRT liposomes, and determine amount of VRT drugs in the two parts, thus ensuring accuracy in determination of encapsulation efficiency.

Conclusion

In summary, the preparation of vinorelbine tartrate is relatively stable process technology with large drug loading, and high encapsulation efficiency. Moreover, there are accurate and convenient methods for measurements of content and encapsulation efficiency.

References

1) Xie Xiangyang, Chen Chen, Lin Wen, Li Yinke, Li Yang, Chen Ying. Preparation and characterization of long-circulating liposomes of vinorelbine tartrate (J). Herald of Medicine, 2015, 11 (03): 379-384.
2) Gong Wei, Zhang Hui, Wang Zhiyuan, Li Bingsheng, Mei Xingguo. Pharmacodynamics and Tissue Distribution of Vinorelbine Tartrate Thermosensitive Liposomes (J). Chinese Pharmaceutical Journal, 2014, 23 (12): 1036- 1039.
3) Yang, Q. and Y.C. Yuan, Analysis of relevant risk factors for intrauterine death of fetuses in the third trimester of pregnancy. Pakistan journal of Pharmaceutical sciences, 2016. 29(6SI): p. 2163-2167.
4) Ji, W.B., et al., Arterial embolization in treatment of hepatic artey pseudoaneurysm. Acta Medica Mediterranea, 2017. 33(3): p. 449-455.
5) Ofori-Kwakye, K., et al., Development and evaluation of natural gum-based extended release matrix tablets of two model drugs of different water solubilities by direct compression. Saudi Pharm J, 2016. 24(1): p. 82-91.
(6) Attari, Z., et al., Enhanced ex vivo intestinal absorption of olmesartan medoxomil nanosuspension: Preparation by combinative technology. Saudi Pharm J, 2016. 24(1): p. 57-63.
7) Gong Wei, Mei Le, Wang Zhiyuan, Yang Ziwei, Di Zhong, Mei Xingguo. Preliminary study on vascular irritation and acute toxicity of vinorelbine tartrate thermosensitive liposomes (J). Pharmaceutical Journal of Chinese People's Liberation Army, 2014, 11 (02): 107-109.
8) Zhang Hongmei, Cui Baiji, Jin Yi. Preparation of vinorelbine tartrate thermosensitive liposomes and its pharmacokinetics in rats (J). Chinese Journal of Pharmaceuticals, 2014, 12 (03): 244-247.
(9) Huang Hesong, Gong Wei, Zhang Hui, Zheng Xiaoli, Mei Xingguo. Preparation of Vinorelbine Tartrate Thermosensitive Liposomes and Measurement of Its Encapsulation Efficiency (J). Chinese Pharmaceutical Journal, 2010, 24 (16): 1250-1254.
10) Ali, M.A., et al., Assessment of biological activity and UPLC-MS based chromatographic profiling of ethanolic extract of Ochradenus arabicus. Saudi Journal Of Biological Sciences, 2016. 23(2): p. 229-236.
11) Singh, G., et al., Unsymmetrically urea silatranes: Synthesis, characterization and a selective on-off fluorescence response to acetate anion. Arabian journal of chemistry, 2017. 10(4): p. 523-531.
12) Yan, J.M., et al., Urodynamic and radiological assessments in sigmoidorectal (Mainz II) pouch for continent urinary diversion in Chinese patients after radical cystectomy. Biomedical research-India, 2017. 28(7): p. 2997-3002.
13) Ge, S., et al., Desulphurization characteristics of bamboo charcoal from sulfur solution. Saudi Journal of Biological Sciences, 2017. 24(1): p. 127-131.
14) Shiri, F., S. Pirhadi and J.B. Ghasemi, Alignment independent 3D-QSAR, quantum calculations and molecular docking of Mer specific tyrosine kinase inhibitors as anticancer drugs. Saudi Pharm J, 2016. 24(2): p. 197-212.
15) Dash, R.N., H. Mohammed and T. Humaira, An integrated Taguchi and response surface methodological approach for the optimization of an HPLC method to determine glimepiride in a supersaturatable self-nanoemulsifying formulation. Saudi Pharm J, 2016. 24(1): p. 92-103.
16) Murtaza, G., et al., Assessment of potential drug-drug interactions and its associated factors in the hospitalized cardiac patients. Saudi Pharm J, 2016. 24(2): p. 220-5.

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