研究简报

包封多酸化合物的固体脂质纳米粒的合成及生物活性研究

杨 勇
(重庆师范大学化学学院,重庆 400047)

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Synthesis and Biological Activity of Solid Lipid Nanoparticles Encapsulated Polyoxometalate

YANG Yong
(*School of Chemistry, Chongqing Normal University, Chongqing* 400047)

Abstract: The solid lipid nanoparticles encapsulated polyoxometalate $K_6[\gamma\text{-}(CpTi)_2SiW_{10}O_{38}]$ (abbreviated as $(CpTi)_2SiW_{10}$ Cp=η^5-C_5H_5) have been prepared and structurally characterized by elemental ananlysis, IR, UV spectroscopy and TEM. The result showed that the polyoxometalate retained the parent structure after being encapsulated and the encapsulation increased the antitumoral activity of the polyoxometalate.

Key words: polyoxometalates; solid lipid nanoparticles; biological activity

0 Introduction

Polyoxometalates (POMs) are early transition metal oxygen anion clusters. Only recently have some of the biological and pharmaceutical properties of POMs been investigated[1,2]. The development of POMs in the medicine has been limited because many derivatives are unstable in water at physiological pH conditions.

Drug carrier system has attracted more and more interest. Since the beginning of the nineties more and more attention has focused on a new drug carrier system——the solid lipid nanoparticles (abbreviated as SLN)[3~6]. SLN has many advantages such as improving the stability, the possibility of controlling drug release and drug targeting.

Stearic acid is used in this work as the matrix and used to combine the drug carrier system with POM for preparation of new POM complex materials, more stable materials at physiological pH. Here we report the preparation of $(CpTi)_2SiW_{10}$ incorporated solid lipid nanoparticles and its antitumoral activity.

1 Materials and methods

1.1 Experimental

The material $K_6[\gamma\text{-}(CpTi)_2SiW_{10}O_{38}]$ was prepared according to the literature[7]. The solid lipid nanoparticles were prepared by warm micro-emulsions. 0.1 g of stearic acid and lecithin were dissolved in 10 mL chloroform and 10 mL acetone, and then the mixture was melted at 70 ℃. A water solution of the material $(CpTi)_2SiW_{10}$ and the surfactant polyoxyethylene 40 stearyl ether was added to the mixture under stirring in order to obtain an optically transparent system. It was kept stirring until the volume of the suspersion was about 5 mL, then the suspersion was immediately

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重庆市教委(No.kj050805),重庆师范大学(No.05XSY001,05XLZ001)资助项目。
*通讯联系人。E-mail:yongtext@163.com;Tel:023-60605665
作者:杨 勇,女,26 岁,硕士研究生,讲师;研究方向:多酸化学及纳米化学。

dispersed in 0~2 ℃ cold water at 1:5 ratio under mechanical stirring for 2 h, thus the POM incorporated solid lipid nanoparticles were obtained. The untrapped POM was separated by dialysis at 4 ℃ for 24 h. The SLN dispersion was then lyophilized with sucrose as cryoprotectant. The dry powder was stored at 4 ℃.

1.2 Physical measurement

Elemental analyses of the POM-SLN were carried out using a Leeman corporation ICP emission spectrometer. IR spectra (2 000~400 cm^{-1}, KBr) of the particles were recorded on a Nicolet Magna 560 IR spectrometer, and UV-Vis (solid) were recorded on a Shimadzu UV-2201 UV-Vis spectrophotometer. Electron micrographs were recorded on a Hitachi (H-600) transmission electron microscope. The particles size ranges were estimated with a 1000 HAS Malvern Zetasizer instrument.

2 Results and discussion

2.1 Elemental analysis results

The elemental analysis results of the $(CpTi)_2SiW_{10}$-SLN show that the composition of the material is W, 1.95%; Si, 0.03%; Ti, 0.10%, and it can be seen that while the POM was being encapsulated by SLN, the ratio of W:Si:Ti was 10:1:2, the same as in its parent, indicating that the POM was not changed during encapsulation.

2.2 Spectra

The IR spectra (Table 1) of the POM incorporated SLN show bands at 980, 950, 900, 870, 810, 750 cm^{-1}, which are characteristic peaks of heteropolyanions with Keggin structure of W-O_d, W-O_b-W, W-O_c-W and Si-O_a. The peak of 1 468 cm^{-1} displays a sharp absorption, which is characteristic of the C-C stretch for a η^5-C_5H_5 ligand bounding to Ti[8]. It was confirmed that the POM was not changed during the process of encapsulation. The UV spectra of POM-SLN show two bands at 202 and 263 nm. The first is assigned to the $O_d \rightarrow W$ charge transfer and the second is assigned to the combination of POM $O_b/O_c \rightarrow W$ charge transfer.

Table 1 IR data of the POM-SLN

Compound	ν_{as}(Si-O_a)	ν_{as}(W-O_d)	ν_{as}(W-O_b-W)	ν_{as}(W-O_c-W)	ν_{as}(C-C)
$(CpTi)_2SiW_{10}$	814	976	948	904	1 468
	752			872	
$(CpTi)_2SiW_{10}$-SLN	810	980	950	900	1 468
	750			870	

2.3 Encapsulate efficiency of the POM-SLN

The encapsulate efficiency equals weight of encapsulated drug (total weight of drug added minus the weight of uncapsulated drug) divided by total weight of drug added × 100%. The weight of the uncapsulated drug was determined by the photometric method. According to the experimental data, the standard curve of $(CpTi)_2SiW_{10}$ at 263 nm is a straight line and the standard curve equation is A =–0.045 13 + 0.006 45C, R= 0.996 1 (N=6).

The determined A value of the uncapsulated $(CpTi)_2SiW_{10}$ is 0.103, from the standard curve equation, C=22.97 μg·mL^{-1}, and the weight of uncapsulated $(CpTi)_2SiW_{10}$ is 11.485 mg with fixed volume of 500 mL. The encapsulation efficiency of $(CpTi)_2SiW_{10}$-SLN=(40 – 11.485) / 40 × 100%=71.3%.

The POM content in the POM-SLN complex is defined as the weight of the POM in the average POM-SLN complex divided by the weight of the average POM-SLN complex. For this case, the content of $(CpTi)_2SiW_{10}$ in the POM-SLN complex=(40 × 71.3%) / (40 × 71.3% +940) × 100%=3.0%.

2.4 Morphology of the POM-SLN

The transmission electron micrograph (Fig.1) shows that the POM incorporated SLN forms relatively uniform nanometer-sized particles and the particles are not affomerated. The size distribution (Fig.2) of the POM-SLN estimated by Zetasizer indicates that the size of the particles is distributed in two uncontinuous limits, in which 80% of the SLN distributes in limit of 245~308 nm with an average diameter of 260.3 nm, and 20% of the SLN distributes in limit of 616~775 nm.

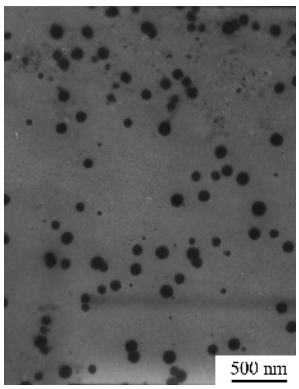

Fig.1　TEM of the $(CpTi)_2SiW_{10}$-SLN

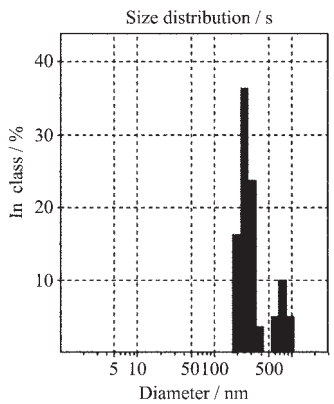

Fig.2　Size distribution of the $(CpTi)_2SiW_{10}$-SLN

2.5 *In vitro* release

In order to investigate the *in vitro* release of the POM incorporated SLN, it was dialyzed in some amounts of phosphate buffer solution (pH 7.4) at 37 ℃ under stirring. The content of the POM in dialyzate was determined by spectrophotometry at certain time intervals. The *in vitro* release profile of $(CpTi)_2SiW_{10}$ from the SLN is shown in Fig.3 and the data is shown in Table 2. It can be seen that the release rate is faster in the initial stage, then becomes slower at 10 h and tends to be basically invariable after 24 h. The result shows that the POM incorporated SLN exhibit an obvious effect of controlled release.

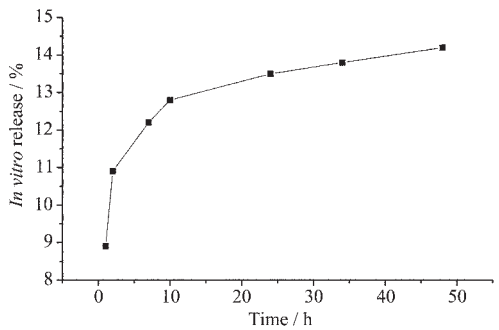

Fig.3　*In vitro* release of the $(CpTi)_2SiW_{10}$-SLN

Table 2　*In vitro* release profile of $(CpTi)_2SiW_{10}$-SLN (%)

1 h	2 h	7 h	10 h	24 h	34 h	48 h
8.9	10.9	12.2	12.8	13.5	13.8	14.2

2.6 Stability of the POM-SLN

Fig.4 is the TEM of the $(CpTi)_2SiW_{10}$-SLN after storage in the refrigerator at 4 ℃ for three months. Comparing with Fig.1, it can be seen that the size and form of the particles are not changed, indicating that the POM incorporated SLN have good stability at 4 ℃.

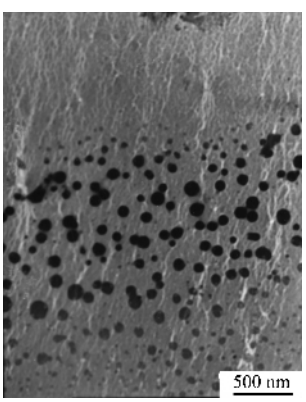

Fig.4　TEM of the $(CpTi)_2SiW_{10}$-SLN after storage for three months at 4 ℃

2.7 Antitumoral activity *in vitro*

In order to evaluate the antitumoral activity of $(CpTi)_2SiW_{10}$-SLN, MTT method[9] (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, also named thiazolyl blue) was used to assay the activity for cancer cells SSMC-7721 *in vitro*. The inhibitory effect and effective cell 50% lethal concentration (IC_{50}) are given in Table 3. From Table 3, one may see that antitumoral activity of POM incorporated SLN is higher than that of the parent $(CpTi)_2SiW_{10}$, indicating that the actural active species is $(CpTi)_2SiW_{10}$.

Table 3 Antitumoral activity of POM and POM-SLN against the liver cancer cells SSMC-7721 *in vitro*

Material	Dose / (μg·mL^{-1})	Concentration of POM[a] / (μg·mL^{-1})	Inhibitory effect / %	IC_{50}[b] / (μg·mL^{-1})
$(CpTi)_2SiW_{10}$	100	100	100	
	10	10	47.3	13.2
	1	1	5.3	
$(CpTi)_2SiW_{10}$-SLN	4 168	125	63	1 933
	2 083	62.5	57	58[c]
	1 042	31.25	13	7.83[d]

[a] The concentration of POM indicates the real content of POM in the POM incorporated solid lipid nanoparticles.

[b] The 50% inhibitory concentration (IC_{50}) is defined as the concentration which suppresses tumor cells by 50%.

[c] The POM concentration in the complex 1 933 × 3.0%=58 μg·mL^{-1}.

[d] The realistic POM concentration reacted on the cancer cells=the POM concentration in the complex × release efficiency of the POM-SLN complex *in vitro* during the time the POM-SLN reacted with the cancer cells. The time is 24 h, and it could be seen that the release efficiency *in vitro* is 13.5% from Table 1, thus 58 × 13.5%=7.83 μg·mL^{-1}.

3 Conclusion

The present study shows clearly the potential of solid lipid nanoparticles as a drug carrier for POMs. The delivery system may open promising possibility for enhancement of the stability and antitumoral activity of POMs.

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