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New polyoxometalate/starch nanomaterial: synthesis, characterization and antitumoral activity

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Using a reversed-phase microemulsion polymerization method, polyoxometalate (POM) $CoW_{11}TiO_{40}$ loaded starch nanoparticles have been prepared and structurally characterized by elemental analyses, IR spectra, UV-vis and ESR spectroscopy. The particle size of $CoW_{11}TiO_{40}$/starch was estimated by transmission electron microscopy (TEM) and the size ranges by a 1000HSA MALVIRN Zetasizer instrument. The results show that the POMs retained the parent structure after encapsulated by starch microspheres, which are able to enhance the stability and antitumoral activity of POMs and also decrease the toxicity of the POM.

Introduction

Polyoxometalates (POMs) have been the subject of many areas of materials science.[1–3] POMs are composed of d^0 cations (mostly commonly Mo^{VI}, W^{VI} or V^{V}) bridged by oxide anions with structures comprised primarily of MO_6 units bridged by one, two or in a few cases three oxygen atoms. These compounds are versatile due to the fact that the properties that dictate their use, including elemental composition, structure, charge density, redox potential, acidity and solubility can be controlled to varying degrees synthetically.[4] Several classes of POMs are known to have a broad spectrum of antiviral and antitumoral properties.[5,6] Despite these promising factors, the development of POMs as antiviral or antitumoral agents has been limited as many derivatives have little or no hydrolytic stability at physiologically relevant pH values (blood serum is typically pH 7.4).

Encapsulation technology represents an area of growing interesting for drug carriers and controlled-release applications. Especially starch-based matrices have afforded promising properties in different areas of encapsulation.[7–9] Lately, methods involving reversed-phase microemulsion polymerization have been described for the preparation of starch microspheres.[10–12] It has been shown that with these methods the encapsulation of several ingredients in starch microspheres is possible, making cost-effective and safe products feasible.

Currently, we are exploring the possibility of combining drug delivery technology and POM syntheses to prepare new POM complex materials in order to solve the problem that most POM compounds are not stable at physiological pH conditions. Herein, we report the preparation of starch loading POM $K_6H_2[CoW_{11}TiO_{40}]\cdot 12H_2O$ ($CoW_{11}Ti$)[13] nanoparticles, and its antitumoral activity and toxicity.

In the synthesis, starch microspheres were prepared from low cost raw soluble starch by reversed-phase microemulsion polymerization methods using phosphorous oxychloride as the linking agent.[12] The titanium polyoxotungstate $CoW_{11}Ti$ loaded starch nanoparticles were prepared by an enveloping method.

Experimental

Preparation of the POM loaded starch particles

1.5 g of soluble starch was added to 10 ml water to form a 15% mixture. The mixture was heated in a boiling water-bath until the mixture turned transparent to form an aqueous starch solution. This aqueous solution was then cooled to room temperature and 200 mg of $K_6H_2[CoW_{11}TiO_{40}]\cdot 12H_2O$[13] was added. This aqueous phase was added dropwise to an oil-phase (containing 200 ml of $C_6H_5CH_3$, 200 ml of $CHCl_3$ and a certain amount of surfactant Span-80) with stirring. It was kept stirring until a microemulsion was formed which was then treated by using sonication for 30 min in order to obtain smaller sized nanostarch particles. To this microemulsion, 3 ml of $POCl_3$ was added and stirring was continued for another 1 h. The microemulsion was left statically to obtain two layers. The water phase was separated and washed with acetone and ethanol to obtain a green solid (1 g), and then the solid was washed with small quantities of water, in order to separate the unencapsulated $CoW_{11}Ti$, until the color of the filtered water changed from green to clear. The solid was dried at 60 °C under vacuum to obtain the dry powder. The $CoW_{11}Ti$ content was analyzed by ICP-absorption spectroscopy.

The time-stability of POM/starch

To determine the stability of $CoW_{11}Ti$ in starch microspheres with time, 0.5 g of a ground sample was placed in different pH buffer solutions: pH 2.0 and pH 7.2, and left in an open environment. After storage periods of 1, 2, 3, 4 and 8 h, the mixtures were centrifuged at 22000*g* for 15 min at room temperature. The supernatant was decanted; the pellets were washed with small amounts of water until the supernatent changed color from blue to white indicating no $CoW_{11}Ti$ was being removed from the starch microspheres. Samples were then analyzed in duplicate for encapsulation efficiency, TEM and ESR spectra.

Physical measurements

The elemental analyses of POM/starch nanoparticles were estimated by a Leeman Plasma Spec (I) ICP-absorption spectrometer. The IR spectra (2000–400 cm^{-1}, KBr) of the particles were recorded on a Nicolet Magna 560 IR spectrometer, and UV-vis (solid) spectra were recorded on a Shimadzu UV-2201 UV-vis spectrophotometer. The transmission electron micrographs were obtained using a Hitachi H-600 Transmission electron microscope. A 1000HSA MALVIRN instrument Zetasizer estimated the size ranges of the particles. ESR spectra were recorded on a JES-Fe 3AX spectrograph at room temperature.

Results and discussion

Elemental contents of the POM/starch complexes

From the elemental analysis results of the POM $CoW_{11}Ti$ loaded starch, the content of the material: W, 8.35; Co, 0.23; Ti, 0.18; K, 0.98%, it can be seen that while the POMs were being encapsulated by starch microspheres, the ratio of W : Co : Ti is

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Table 1 Cyclic voltammetry data for $CoW_{11}Ti$ and $CoW_{11}Ti$/starch (mV) [a b]

pH	2.0	3.2	4.7	5.2	5.7	6.2	6.7	7.2
$E_{pc}(1)$			−570	−608	−640	−676	−708	
$E_{pa}(1)$			−540	−570	−601	−634	−661	
$E_{pc}(2)$			−728	−756	−800	−842	−876	
$E_{pa}(2)$			−690	−716	−759	−801	−833	
$E_{pc}(3)$			−812	−846	−890	−942	−992	
$E_{pa}(3)$			−780	−815	−867	−909	−945	
$E_{p'c}(1)$	−400	−471	−570	−606	−643	−676	−701	−744
$E_{p'a}(1)$	−382	−439	−538	−562	−601	−633	−670	−711
$E_{p'c}(2)$	−558	−618	−727	−756	−812	−846	−870	−913
$E_{p'a}(2)$	−521	−582	−690	−710	−763	−806	−838	−872
$E_{p'c}(3)$	−639	−738	−810	−842	−893	−942	−990	−1031
$E_{p'a}(3)$	−610	−716	−783	−813	−861	−908	−940	−987

[a] Scan speed 100 mV s^{-1}. [b] E_{pc} and E_{pa}, $E_{p'c}$ and $E_{p'a}$ represent the redox couples corresponding to $CoW_{11}Ti$ and $CoW_{11}Ti$ /starch, respectively.

11 : 1 : 1, as in its parent, indicating that the $CoW_{11}Ti$ did not change during reaction. According to the elemental analysis results, it can be calculated that the content of $CoW_{11}Ti$ in the starch microspheres is 13.2%. From this, and the amounts of $CoW_{11}Ti$ and starch used in the experiment, the encapsulation efficiency of starch microspheres towards the POM $CoW_{11}Ti$ is 61%.

Spectra

The IR spectra of the POM/starch nanoparticles show bands at 3595, 1159, 1003, 949, 933, 875, 762 and 685 cm^{-1}, four of which are characteristics of W–O_d, W–O_b–W, W–O_c–W and Co–O_a asymmetric stretching vibrational peaks (949, 933, 875, 762, 685 cm^{-1}) as shown [13] in heteropolyanions with a Keggin structure, suggesting that the POMs in starch microspheres also retain the Keggin structure and showing that the POM $CoW_{11}Ti$ does not change during the experiment. Comparing the IR spectra of the nanoparticles with that of the parent $CoW_{11}Ti$, the vibrational frequencies do not change. Therefore, it is confirmed that a simple physical absorption, not a chemical interaction, exists between the POM and the starch microspheres. On comparison of the IR spectra of $CoW_{11}Ti$ with that of $CoW_{11}Ti$/starch, a new C–O–P vibration peak is seen at 1158 cm^{-1}, indicating that during the starch reacting with $POCl_3$, the cross-linking reaction occurred. The P atom content in $CoW_{11}Ti$/starch microspheres (1.45%) also confirms this point.

The solid UV-vis spectrum of $CoW_{11}Ti$/starch shows two bands at 260 and 625 nm. The first band is assigned to the combination of POM $O_{b,\,c}$–W charge transfer with the band of starch microspheres. The visible spectrum of $CoW_{11}Ti$/starch shows a broad envelope centered at 625 nm in which three maxima are visible, which is the same as in the parent $CoW_{11}Ti$. This pattern is typical for tetrahedral cobalt(II) complexes [14] and can be assigned to spin-allowed transitions to an excited quartet state $^4T_1(p) \longleftarrow {}^4A_2(F)$. The ESR spectrum of $CoW_{11}Ti$/starch at room temperature shows two signals for $CoW_{11}Ti$. The first is a strong asymmetric signal with $g = 5.01$ assigned to $CoW_{11}Ti$, typical for high-spin tetrahedral cobalt(II) (CoO_4). The second signal is a weak and unassigned hyperfine signal with $g = 2.01$ for $CoW_{11}Ti$. The visible and ESR spectra of $CoW_{11}Ti$/starch confirm that the POMs did not change during the encapsulation.

The transmission electron micrographs (TEM) (Fig. 1) show that the POM/starch forms relatively uniform nanometer sized particles with average diameter of about 10–15 nm; the shape of the nanoparticles is spherical and the particles did not agglomerate. The size-distribution estimated from the Zatesizer is from 20 to 60 nm, with a maximum in the particle distribution histogram at 35 nm. The reason why the average diameter obtained by TEM is smaller than that from size-distribution is that generally the outer covering of starch microspheres were contracted by the covering agent used in the TEM observation.

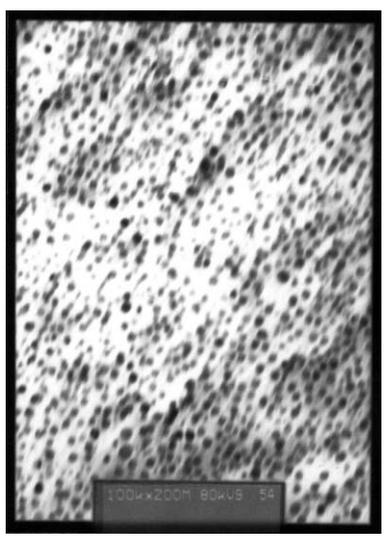

Fig. 1 TEM of $CoW_{11}Ti$/starch.

The stability of $CoW_{11}Ti$/starch

In order to assess the stability of $CoW_{11}Ti$ in the starch microspheres under different pH conditions, we assayed this by cyclic voltammetry (Table 1) of the POM/starch complex in the pH range 2.0–7.2. It can be seen that for the POM/starch microspheres, the redox potentials do not change in the region pH 4.7–6.7, showing that the POM compound does not change after being encapsulated. Additionally the shapes of redox waves of the microspheres at pH 2.0–4.7 do not change showing that the POM $CoW_{11}Ti$ is stable at these pH regions and its limit of pH-stability in water extends from pH 4.7–6.7 to pH 2.0–7.2.

The elemental analysis results of the experiments assessing the time-stability of $CoW_{11}Ti$ /starch in this study are shown in Table 2. It can be seen that at pH 2.0, the $CoW_{11}Ti$ does not change its content within 2 h as the ratio of W : Co : Ti is constant at 11 : 1 : 1, the same as its parent except there is some $CoW_{11}Ti$ release from the starch microspheres. The ESR spectra of the sample show two signals similarly to the parent $CoW_{11}Ti$, at $g_1 = 5.10$ and $g_2 = 2.15$, indicating that the $CoW_{11}Ti$ retains its structure when encapsulated by the starch microspheres; TEM shows that the sizes and the shapes of the POM/starch nanoparticles do not change, indicating that $CoW_{11}Ti$ is stable, protected by the starch. However, beyond 2 h, the POMs decompose into cobalt or titanium tungstate and some insoluble isopolytungtate acid while the starch decomposes into poly-, di- or mono-saccharides. The TEM spectra show that some crystal salts appear and the shape of the starch microspheres also change. At pH 7.4, it can be seen from the elemental analysis results that the POMs remain intact for about 4 h with some leaking from the starch microspheres, and

Table 2 The elemental analysis results of $CoW_{11}Ti$/starch in stability experiments (%)

	Content (%)											
	pH 2.0						pH 7.4					
	0 h	1 h	2 h	3 h	4 h	8 h	0 h	1 h	2 h	3 h	4 h	8 h
W	8.35	7.65	5.11	3.29	2.78	0.00	8.35	7.89	6.52	4.96	4.02	0.04
Co	0.28	0.22	0.16	0.04	0.00	0.00	0.28	0.24	0.18	0.13	0.10	0.00
Ti	0.18	0.18	0.13	0.02	0.00	0.00	0.18	0.19	0.15	0.12	0.09	0.00
W : Co : Ti	11 : 1 : 1	11 : 1 : 1	11 : 1 : 1				11 : 1 : 1	11 : 1 : 1	11 : 1 : 1	11 : 1 : 1	11 : 1 : 1	
Content of POM in starch (%)	13.2	12.2	8.1				13.2	12.6	10.4	7.9	6.4	

Table 3 The antitumoral activity of the nanoparticles against Hela and HL-60 cell lines

			Hela		HL-60	
Material	Dose/μg mL^{-1}	Concentration of POM[a]/μg mL^{-1}	Inhibitory effect (%)	IC_{50}[b] /μg mL^{-1}	Inhibitory effect (%)	IC_{50}/μg mL^{-1}
$CoW_{11}Ti$/starch	200	26.4	100.0		100	
	100	13.2	100.0		100.0	
	10	1.3	36.3		20.2	
	5	0.6	16.2	28.9 (3.81[c])	3.6	46.7 (6.16[c])
$CoW_{11}Ti$	100	100	91.7		90.4	
	50	50	69.4		76.2	
	10	10	28.1	19.8	29.6	20.9
Starch			1.02			
			1.00			

[a] The concentration of POM indicates the content of $CoW_{11}Ti$ in the POM/starch complex. [b] The 50% inhibitory concentration (IC_{50}) is defined as the concentration which suppresses tumor cells by 50%. [c] The $CoW_{11}Ti$ concentration in starch microspheres.

beyond 4 h the $CoW_{11}Ti$ decomposes into simple anions or heteropolyanions with vacancies. ESR also shows two signals with $g_1 = 5.21$, $g_2 = 2.20$, indicating that in this condition the POMs are stable. The result of TEM and ESR of the situation at pH 7.4 and 8 h show that POMs decompose, complementary to the elemental analysis results.

Antitumoral activity

In order to evaluate the antitumoral activity of POM/starch nanoparticles, MTT[15] (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide(Thiazolyl Blue) was used to assay the activity for HL-60 and Hela cancer cells *in vitro*. The inhibitory effect and effective cell 50% lethal concentration (IC_{50}) are given in Table 3.

From Table 3, it can be seen that the antitumoral activity of POM/starch nanoparticles is higher than that of the parent $CoW_{11}Ti$ and starch, indicating that the actual active species is $CoW_{11}Ti$. In order to estimate the effect of starch on antitumoral activity, a cellular penetration test was carried out. Hela cells with PBS (phosphate buffer solution) containing 13.2 μg $CoW_{11}Ti$ or 100 μg $CoW_{11}Ti$/starch (this complex containing 13.2 μg $CoW_{11}Ti$) were incubated in a 5% CO_2 incubator for 24 h. After the supernant was removed, the mixtures were then treated with HNO_3 and $CHCl_3$, and the contents of W were determined by ICP absorption spectroscopy. The contents of W in the $CoW_{11}Ti$ and $CoW_{11}Ti$/starch expriments were 2.13 μg and 6.94 μg, respectively, corresponding to $CoW_{11}Ti$ contents of 3.37 μg and 10.97 μg, respectively. Thus, the penetrating efficiencies are 25.52% and 83.11% for $CoW_{11}Ti$ and $CoW_{11}Ti$/starch particles, respectively. This result shows that the POM/starch nanoparticles can penetrate into cells more easily than the POM compound alone, indicating that the starch can help $CoW_{11}Ti$ penetrate easily into cancer cells, so the POM/starch complex exhibits higher antitumoral activity than its parent POM compound.

In order to assess the toxicity of the POM/starch complex, starch and $CoW_{11}Ti$, a cellular toxicity test was carried our with the Hela cells (1×10^5) containing the three different materials incubated for 24 h, and the cell survival rates was then calculated to determine the toxicity of the complexes. The survival rates are 67%, 96–100% and 38%, respectively, showing that the $CoW_{11}Ti$ encapsulated by starch has less cell toxicity *in vitro* than its parent $CoW_{11}Ti$.

The present study clearly shows the potential of starch as a drug carrier for POMs. This delivery system opens promising possibility for enhancement of the stability and antitumoral activity of POMs and coupled with the decreased toxicity of the POMs makes these results very interesting. This suggests that POM/starch complexes are promising and suitable devices for antiviral and antitumoral drug applications.

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