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Encapsulation and Stabilization of Polyoxometalates in Self-Assembled Supramolecular Hydrogels

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Received 00th January 2012,
Accepted 00th January 2012
DOI: 10.1039/x0xx00000x
www.rsc.org/

We have encapsulated the polyoxoanions \([P_2W_{18}O_{62}]^{6-}\) and \([P_2W_{13}V_5O_{60}]^{9-}\) in a self-assembled carboxy-methyl-chitosan (CMC) hydrogel, exhibiting a regular super-structure in water at physiological pH. We performed stability studies as a function of temperature and polyoxometalate (POM) loading, and observed exceptional \(T_{gel}\) properties. This work is a step forward towards developing biologically active polyoxometalate-based materials.

Hydrogels can be tuned at the molecular level and allow for the synthesis of tailor-made “smart hydrogels” with desired properties. Such hydrogels can be triggered by external stimuli, such as pH, temperature, enzyme/ionic strength, electric and magnetic fields, biomolecules/biomaterials and light, to break the superstructure and release the encapsulated drug at a specific target. Using this feature of hydrogels, one can tune the final architecture of the hydrogel and use such “smart materials” for selected applications, for example sustained/targeted drug delivery. On the other hand, in the last three decades POMs have been tested for biomedical applications such as antiviral (in particular anti-HIV), antinfluenza, antibacterial, anticancer, antidiabetic, and recently anti-Alzheimer. A major issue preventing the prolonged use of POMs in mammals has been the lack of selectivity, and hence the resulting toxicity. In recent years there have been some scarce reports indicating reduced POM toxicity and high cellular uptake, but the real way forward appears to be targeted and controlled delivery of POMs using biodegradable/biocompatible hydrogels. In fact, a careful literature survey reveals that some efforts have been made already in this direction, trying to minimize the toxicity of POMs by encapsulating them in biomaterials such as CMC or its derivatives. Suppression of HeLa tumour cells by trimethyl- and CMC nanocomposites containing POM ([CoW11O39]6−) has been reported. Also other applications have been investigated, using carbon nanotube assisted high loading and controlled release of POMs in biodegradable multilayer thin films. The antitumour activity of \(\{Ti_5SiW_{15}\}\) in starch nanoparticles was demonstrated, inhibiting the growth of HL-60 and HeLa tumour cell lines and in vivo studies also suppressed tumour in C57. The toxicity of POMs was reduced when liposome-encapsulated POMs were employed against HL-60 tumours in vivo and based upon in vitro measurements with KB and HeLa cancer cells. POMs retain their parent structure after being encapsulated by liposome, and liposomal encapsulation increases the anti-tumour activity of POMs. Thus, the use of POMs in ways that reduces their toxicity might be a strategy for preparing promising drugs. To the best of our knowledge, no study on the use of \([\alpha-P_2W_{18}O_{62}]^{6-}\) and \([P_2W_{13}V_5O_{60}]^{9-}\) in hydrogel formation with CMC has been reported yet.

Herein, we report a POM-assisted, pH-dependent, reversible, self-assembled hydrogel formation (Scheme 1), its stability study and unexpected \(T_{gel}\) properties. We encapsulated the two model antidiabetic polyoxanions \([P_2W_{18}O_{62}]^{6-}\) \((P_2W_{18})\) and its tri-vanadium derivative \([P_2W_{13}V_5O_{60}]^{9-}\) \((P_2W_{13}V_3)\) in CMC hydrogel (Scheme 1).

The hydrogels CMC-P2W18 and CMC-P2W13V3 were prepared by simple mixing of an aqueous solution of CMC and adding the respective solid POM salt, followed by stirring with a glass rod (see Exp. Section). The water content of the CMC-P2W18 and CMC-P2W13V3 hydrogels were 96.0% and 96.5%, respectively, as determined by TGA/DSC (see Figs. S1 and S2). No gel formation was observed for CMC alone, but in the presence of the polyoxanions. For polyoxanion \(P_2W_{18}\) the gel formation was fast at pH 9.1, whereas
Scheme 1. pH dependent formation of self-assembled hydrogels.

In the case of $P_2W_{15}V_3$ no gel was formed at such pH, but rather at pH 7.4, and after a longer stirring time.

In case of CMC-$P_2W_{18}$ gel we observed a bluish colour appearing after 15 min at pH 7 (Figure 1, inset B). Interestingly, the blue colour appeared after 30 min at pH 8, and after 1 h at pH 9.1. It appears likely that the blue colour indicates formation of heteropoly blue species (i.e. reduced POMs)\(^2\), which in turn suggests oxidation of CMC. However, the quantity was too small for detection by IR. We also monitored the stability of $P_2W_{18}$ in the CMC gel by \(^{31}\)P NMR spectroscopy, but this experiment took minimum 1 h, due to the low POM concentration in the gel. After 1 h the \(^{31}\)P NMR spectrum of CMC-$P_2W_{18}$ showed 2 singlets at -7.4 and -14.6 ppm (Figure 1, inset A), suggesting transformation of the plenary Wells-Dawson ion to the monolacunary $[P_2W_{17}O_{68}]^{10-}$. For comparison, the \(^{31}\)P NMR of $P_2W_{18}$ shows a singlet at -13.1 ppm (Fig. S3) in aqueous solution, and for $[P_2W_{17}O_{68}]^{10-}$ in aqueous solution two singlets at -9.0 and -13.1 ppm are observed.\(^2\)

Figure 1. Temperature and POM-loading dependent gelling properties of CMC-$P_2W_{18}$. Inset A shows the \(^{31}\)P NMR spectrum of the gel after 1 h, and inset B shows a picture of the actual gel in a vial.

The CMC-$P_2W_{15}V_3$ hydrogel of the trivanadium-based POM formed a yellow gel at physiological pH 7.4, which was stable for more than 48 hours, as based on \(^{31}\)P NMR (Figure 2, inset A). Two signals at -6.9 and -14.6 ppm were observed for the hydrogel, which is identical to the shifts of the same polyanion in aqueous solution (-6.9 and -14.6 ppm, see Fig. S4). The yellow gel slowly changed colour to green (Fig. S5), indicative of partial reduction of the polyanion, but to a much smaller degree compared to $P_2W_{18}$ (vide supra). Interestingly, $P_2W_{15}V_3$ did not form a gel between pH 8 – 9, but rather remained in solution (Fig. S6), which in fact became even less viscous compared to the original CMC solution.

![Figure 2. Temperature and POM-loading dependent gelling properties of CMC-$P_2W_{18}$. Inset A shows the \(^{31}\)P NMR spectrum of the gel after 52 h, and inset B shows a picture of the actual gel in a vial.](image)

In essence, both polyanions $P_2W_{18}$ and $P_2W_{15}V_3$ form gels in the presence of CMC, but under slightly different pH conditions. In the absence of POM the CMC solution remains clear and viscous even after prolonged stirring (Fig. S7). The presence of the POMs in the hydrogel is supported by analysis of the xerogels (dried gels) by FT-IR spectroscopy (Figs. S8 and S9), which shows peaks corresponding to CMC as well as to POM. At pH 6, gel formation was observed for both POMs, but the gel decomposed leading to a non-transparent CMC-POM composite material.

The peculiar pH dependence of gel formation for $P_2W_{18}$ and $P_2W_{15}V_3$ (vide supra) prompted us to determine the gelation temperature ($T_{gel}$) of these hydrogels by the ball drop method. We observed for $P_2W_{18}$ that $T_{gel}$ increased linearly from 35 to 85 °C (Figure 1) with an increase in percent POM loading (10 to 50%), whereas for $P_2W_{15}V_3$ the $T_{gel}$ remained constant between 80 to 90 °C independent of POM loading (Figure 2). Reversibility of these gels was observed after checking $T_{gel}$ and confirming once again the gel formation at room temperature (Figs. S5 and S10).

The observed gelling differences for $P_2W_{18}$ and $P_2W_{15}V_3$ must be due to differences in composition and charge of the polyanions, as both are isostructural (Wells-Dawson structure). The more negatively charged $P_2W_{15}V_3$ is known to be hydrolytically more stable at physiological pH than $P_2W_{18}$, and probably forms stronger
electrostatic interactions with the cationic CMC. On the other hand, the less negatively charged P3W18 forms a gel with a superstructure becoming stronger with increasing POM loading.

**Experimental details:**

CMC18 and both polyanions P3W1826 and P3W18V27 were synthesized as reported in the literature. CMC (33.33 mg) was dissolved in 1 ml distilled water and the pH of this colourless, transparent, viscous solution was 9.1. The CMC-P3W18 hydrogel was prepared by adding solid P3W18 (16.66 mg) to 1 ml of the CMC solution, and stirred by a glass rod until a transparent, green, self-assembled hydrogel is obtained. The CMC-P3W18V3 hydrogel was prepared by following the same procedure, just replacing P3W18 by P3W18V3, but at pH 7.4, and more stirring time is required. Dilute hydrochloric acid was used for pH adjustment. The FT-IR spectra of both hydrogels were recorded on a Thermo Scientific Nicolet-6700 instrument, 31P NMR measurements were performed directly on the hydrogels in 5 mm tubes using a Bruker DRX 400 instrument, and TGA/DSC data were recorded using a Mettler-Toledo TGA/DSC-1 Star system.

**Experimental Procedure (Ball Drop Method):**

3 ml gel was prepared in a 15 ml test tube following the above-mentioned procedure. A glass ball weighing 358 mg was placed gently on the surface of the gel. The test tube was then kept in a water bath, where the temperature was set to increase linearly. Over the course of time with increasing temperature, the glass ball penetrated through the gel and when the glass ball touched the bottom of the test tube the temperature was recorded as Tgel. The reversibility of this gelling phenomenon upon cooling was also studied and as expected we observed gel formation at room temperature. The glass ball remained trapped at the bottom of the inverted test tube for days, confirming reversibility of gel reformation.

**Conclusions**

In conclusion, we have demonstrated that the two isostructural Wells-Dawson polyanions [P3W18O62]6- and [P3W18V3O62]5-b form gels with CMC in a reversible fashion. The hydrogels CMC-P3W18 and CMC-P3W18V3 were prepared by simple mixing of an aqueous solution of CMC and adding the respective solid POM salt. Interestingly, the hydrogel CMC-P3W18V3 is formed only at pH 7.4, whereas the hydrogel CMC-P3W18 is formed between pH 7 - 9. However, CMC-P3W18V3 is stable for a much longer time than CMC-P3W18 (48 h vs 1 h). In addition, the gelling temperature Tgel for the former is fairly independent of POM loading, unlike the latter. The detailed reasons for such behaviour are not yet clear, but this will be examined in future work. We plan to investigate a family of polyanions, systematically changing shape, size, charge and composition, in order to extract which of these parameters is dominant for gel formation. Our results demonstrate potential for tuning the release pattern of POMs in future biomedical applications.

**Notes and references**

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VP and SJ acknowledge Department of Science and Technology (DST), Ministry of Science and Technology (MoST), Government of India (GOI) for financial assistance from Fast-Track Scheme No. SR/FT/CS-133/2011.

Electronic Supplementary Information (ESI) available: [Experimental procedure, Figures, Pictures and TGA/DSC of hydrogels, 31P NMR and FT-IR spectra]. See DOI: 10.1039/c000000x/


Polyoxometalate assisted, pH dependent self-assembled hydrogel formation.

Random CMC Fibrils in aqueous solution + POM → Systematic super-structure formation

With $P_2W_{18}$ Gel formation at pH 9.1/8.0/7.0, stability in air 1 Hr.
With $P_2W_{15}V_3$ Gel formation only at pH 7.4, stability in air 48 Hrs.

$P_2W_{18}$ Gel $P_2W_{15}V_3$ Gel