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Encapsulation and Stabilization of Polyoxometalates in Self-Assembled Supramolecular Hydrogels

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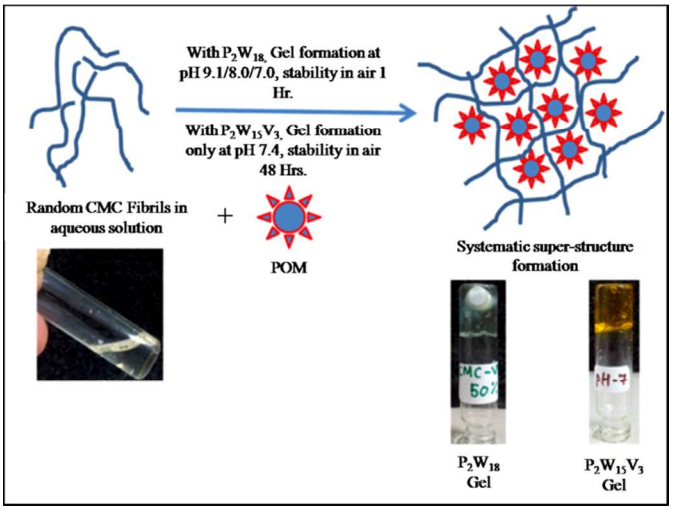
We have encapsulated the polyoxoanions $[P_2W_{18}O_{62}]^{6-}$ and $[P_2W_{15}V_3O_{62}]^{9-}$ in a self-assembled carboxy-methyl-chitosan (CMC) hydrogel, exhibiting a regular super-structure in water at physiological pH. We performed stability studies as a function of temperature and polyoxometalate (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,[1] temperature,[2] enzyme/ionic strength,[3] electric[4] and magnetic fields,[5] biomolecules/biomaterials[6] and light,[7] to break the superstructure and release the encapsulated drug at a specific target.[8] 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.[9] On the other hand, in the last three decades **POMs** have been tested for biomedical applications such as antiviral[10] (in particular anti-HIV),[11] antiinfluenza,[12] antibacterial,[13] anticancer,[14] antidiabetic,[15] and recently anti-Alzheimer.[16]

A major issue preventing the prolonged use of **POMs** in mammals has been the lack of selectivity, and hence the resulting toxicity.[17] 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.[18] Suppression of HeLa tumour cells by trimethyl- and **CMC** nanocomposites containing **POM** ($[CoW_{11}TiO_{40}]^{8-}$) has been reported.[19] Also other applications have been investigated, using carbon nanotube assisted high loading and controlled release of **POMs** in biodegradable multilayer thin films.[20,21] The antitumour activity of $\{Ti_6Si_2W_{18}\}$ 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.[22] 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.[23] **POMs** retain their parent structure after being encapsulated by liposome, and liposomal encapsulation increases the anti-tumour activity of **POMs**.[24] 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 $[\alpha\text{-}P_2W_{18}O_{62}]^{6-}$ and $[P_2W_{15}V_3O_{62}]^{9-}$ 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 polyanions $[P_2W_{18}O_{62}]^{6-}$ (**P_2W_{18}**) and its tri-vanadium derivative $[P_2W_{15}V_3O_{62}]^{9-}$ (**$P_2W_{15}V_3$**) in **CMC** hydrogel (Scheme 1).

The hydrogels **CMC-P_2W_{18}** and **CMC-$P_2W_{15}V_3$** 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-P_2W_{18}** and **CMC-$P_2W_{15}V_3$** 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 polyanions. For polyanion **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 $\mathbf{P_2W_{15}V_3}$ no gel was formed at such pH, but rather at pH 7.4, and after a longer stirring time.

In case of **CMC-$\mathbf{P_2W_{18}}$** 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**),[25] which in turn suggests oxidation of **CMC**. However, the quantity was too small for detection by IR. We also monitored the stability of $\mathbf{P_2W_{18}}$ 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-$\mathbf{P_2W_{18}}$** 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 $[P_2W_{17}O_{61}]^{10-}$. For comparison, the ^{31}P NMR of $\mathbf{P_2W_{18}}$ shows a singlet at -13.1 ppm (Fig. S3) in aqueous solution, and for $[P_2W_{17}O_{61}]^{10-}$ in aqueous solution two singlets at -9.0 and -13.1 ppm are observed.[25]

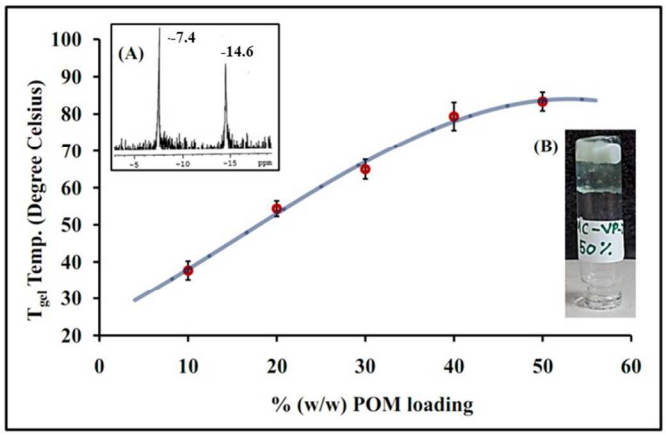


Figure 1. Temperature and **POM**-loading dependent gelling properties of **CMC-$\mathbf{P_2W_{18}}$**. Inset A shows the ^{31}P NMR spectrum of the gel after 1 h, and inset B shows a picture of the actual gel in a vial.

The **CMC-$\mathbf{P_2W_{15}V_3}$** 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 $\mathbf{P_2W_{18}}$ (*vide supra*). Interestingly, $\mathbf{P_2W_{15}V_3}$ 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.

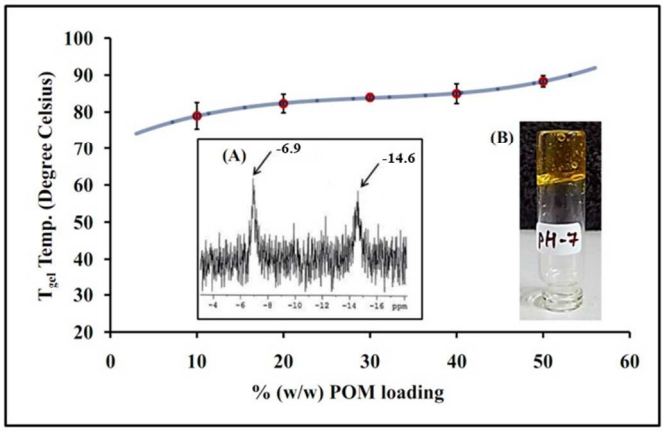


Figure 2. Temperature and **POM**-loading dependent gelling properties of **CMC-$\mathbf{P_2W_{15}V_3}$**. Inset A shows the ^{31}P NMR spectrum of the gel after 52 h, and inset B shows a picture of the actual gel in a vial.

In essence, both polyanions $\mathbf{P_2W_{18}}$ and $\mathbf{P_2W_{15}V_3}$ 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 $\mathbf{P_2W_{18}}$ and $\mathbf{P_2W_{15}V_3}$ (*vide supra*) prompted us to determine the gelation temperature (T_{gel}) of these hydrogels by the ball drop method. We observed for $\mathbf{P_2W_{18}}$ that T_{gel} increased linearly from 35 to 85 °C (Figure 1) with an increase in percent **POM** loading (10 to 50%), whereas for $\mathbf{P_2W_{15}V_3}$ 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 $\mathbf{P_2W_{18}}$ and $\mathbf{P_2W_{15}V_3}$ must be due to differences in composition and charge of the polyanions, as both are isostructural (Wells-Dawson structure). The more negatively charged $\mathbf{P_2W_{15}V_3}$ is known to be hydrolytically more stable at physiological pH than $\mathbf{P_2W_{18}}$, and probably forms stronger

electrostatic interactions with the cationic **CMC**. On the other hand, the less negatively charged $\mathbf{P_2W_{18}}$ forms a gel with a superstructure becoming stronger with increasing **POM** loading.

Experimental details:

CMC[18a] and both polyanions $\mathbf{P_2W_{18}}$[26] and $\mathbf{P_2W_{15}V_3}$[27] 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-$\mathbf{P_2W_{18}}$** hydrogel was prepared by adding solid $\mathbf{P_2W_{18}}$ (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-$\mathbf{P_2W_{15}V_3}$** hydrogel was prepared by following the same procedure, just replacing $\mathbf{P_2W_{18}}$ by $\mathbf{P_2W_{15}V_3}$, 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, ^{31}P 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^e system.

T_{gel} 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 T_{gel}. 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 isostructrural Wells-Dawson polyanions $[\mathbf{P_2W_{18}O_{62}}]^{6-}$ and $[\mathbf{P_2W_{15}V_3O_{62}}]^{9-}$ form gels with **CMC** in a reversible fashion. The hydrogels **CMC-$\mathbf{P_2W_{18}}$** and **CMC-$\mathbf{P_2W_{15}V_3}$** were prepared by simple mixing of an aqueous solution of **CMC** and adding the respective solid **POM** salt. Interestingly, the hydrogel **CMC-$\mathbf{P_2W_{15}V_3}$** is formed only at pH 7.4, whereas the hydrogel **CMC-$\mathbf{P_2W_{18}}$** is formed between pH 7 - 9. However, **CMC-$\mathbf{P_2W_{15}V_3}$** is stable for a much longer time than **CMC-$\mathbf{P_2W_{18}}$** (48 h vs 1 h). In addition, the gelling temperature T_{gel} 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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Electronic Supplementary Information (ESI) available: [Experimental procedure, Figures, Pictures and TGA/DSC of hydrogels, ^{31}P NMR and FT-IR spectra]. See DOI: 10.1039/c000000x/

1 H. Zhang, Y. Dong, L. Wang, G. Wang, J. Wu, Y. Zheng, H. Yang and S. Zhu, *J. Mater. Chem.* 2011, **21**, 13530.
2 L. Klouda and A. G. Mikos, *Eur. J. Pharm. Biopharm.* 2008, **68**, 34.
3 X. Li, Y. Gao, Y. Kuang and B. Xu, *Chem. Comm.* 2010, **46**, 5364.
4 R. V. Kulkarni and S. Biswanath, *J. Appl. Biomater. Biomech.* 2007, **5**, 125.
5 X. H. Zhao, J. Kim, C. A. Cezar, N. Huebsch, K. Lee, K. Bouhadir D. and J. Mooney, *Proc. Natl. Acad. Sci.* USA, 2011, **108**, 67.
6 Z. Yang and B. Xu, *J. Mater. Chem.* 2007, **17**, 2385.
7 D. D. Diaz, E. Morin, E. M. Schon, G. Budin, A. Wagner and J. S. Remy, *J. Mater. Chem*. 2011, **21**, 641.
8 a) L. Zha, B. Banik and F. Alexis, *Soft Matter,* 2011, **7**, 5908; b) G. M. Soliman, A. Sharma, D. Maysinger and A. Kakkar, *Chem. Comm.* 2011, **47**, 9572; c) J. W. Steed, *Chem. Comm*. 2011, **47**, 1379.
9 F. Brandl, F. Kastner, R. M. Gschwind, T. Blunk, J. Tebmar and A. Opferich, *J. Controlled Release,* 2010, **142(2)**, 221.
10 a) D. -L. Long, R. Tsunashima and L. Cronin, *Angew. Chem. Int. Ed,* 2010, **49**, 1736; b) A. Müller, F. Peters, M. T. Pope and D. Gatteschi, *Chem. Rev.* 1998**, 98**, 239.
11 a) B. Hasenknopf, *Front. Biosci.* 2005, **10**, 275; b) S. Shigeta, S. Mori, T. Yamase and N. Yamamoto, *Biomed. Pharmacother.* 2006, **60**, 211; c) J. P. Berry and P. Galle, *Exp. Mol. Pathol,* 1990, **53**, 255.
12 S. M. Hosseini, E. Amini, M. T. Kheiri, P. Mehrbod, M. Shahidi and E. Zabihi, *Int. J Mol. Cell Med Winter,* 2012, **1(1)**, 21.
13 a) D. Chen, J. Peng, H. Pang, P. Zhang, Y. Chen, Y. Shen, C. Chen and H. Ma, *Z. Naturforsch*. 2010, **65b**, 140; b) M. Inoue, K. Segawa, S. Matsunaga, N. Matsumoto, M. Oda and T. Yamase, *J. of Inorg. Biochem.* 2005, **99,** 1023.
14 a) Z. Dong, R. Tan, J. Cao, Y. Yang, C. Kong, J. Du, S. Zhu, Y. Zhang, J. Lu, B. Huang and S. Liu, *Eur. J. of Med. Chem.* **2011**, *46*, 2477; b) C. Li, J. Lu, F. Tu, J. Chen and Y. Li, *Inorg. Chem. Comm.* 2011, **14**, 1192.

15 a) Y. Schechter and S. J. D. Karlish, *Nature,* 1980, **284**, 556; b) C. E. Heyliger, A. G. Tahiliani and J. H. McNeill, *Science,* 1985, **227**, 144; c) D. C. Crans, J. J. Smee, E. Gaidamauskas, L. Lang, *Chemical Reviews,* 2004, **104**, 849.

16 a) N. Gao, H. Sun, K. Domg, J. Ren, T. Duan, C. Xu and X. Qu, *Nature Communications*, 2014, **5,** 3244; b) J. Iqbal, M. Barsukova-Stuckart, M. Ibrahim, S. U. Ali, A. A. Khan and U. Kortz, *Med. Chem. Res*. 2013, **22**, 1224; c) C. E. Müller, J. Iqbal, Y. Baqi, H. Zimmermann, A. Röllich and H. Stephan, *Bioorg. Med. Chem. Lett,* 2006, **16**, 5943; d) J. Geng, M. Li, J. Ren, E. Wang and X. Qu, *Angew. Chem. Int. Ed,* 2011, **123**, 4270; e) J. E. Kim and M. Lee, *Biochem. Biophys. Res. Comm.* 2003, **303**, 576

17 J. Fischer, L. Ricard and R. Weiss, *J. Am. Chem. Soc.* 1976, ***98*,** 3050

18 a) G. Geisberger, S. Paulus, M. Carraro, M. Bonchio and G. R. Patzke, *Chem. Eur. J.* 2011, **17**, 4619; b) T. Meibner, R. Bergmann, J. Oswald, K. Rode, S. Holger, W. Richter, H. Zanker, W. Kraus, F. Emmerling and G. Reck, *Trans. Met. Chem.* 2006, **31**, 603.

19 G. Geisberger, E. B. Gyenge, C. Maake and G. R. Patzke, *Carbohydrate Polymers*, 2013, **91**, 58.

20 a) V. Ball, C. Ringwald, J. Bour, M. Michel, R. Al-Oweini and U. Kortz, *Journal of Colloid and Interface Science*, 2013, **409**, 166; b) V. Ball, M. Barsukova-Stuckart and U. Kortz, *Colloid and Polymer Science*, 2013, **291(5)**, 1219; c) G. Ladam, V. Toniazzo, D. Ruch, H. Atmani, M. Ibrahim, U. Kortz and V. Ball, *RSC Advances*, 2012, **2**, 3700; d) V. Ball, F. Bernsmann, S. Werner, J. C. Voegel, L. F. Piedra-Garza and U. Kortz, *European Journal of Inorganic Chemistry*, 2009*,* **34**, 5115.

21 Q. Zhao, X. Feng, S. Mei and Z. Jin, *Nanotechnology*, 2009, **20**, 1.

22 F. Zhai, D. Li, C. Zhang, X. Wang and R. Li, *European Journal of Medicinal Chemistry*, 2008, **43**, 1911.

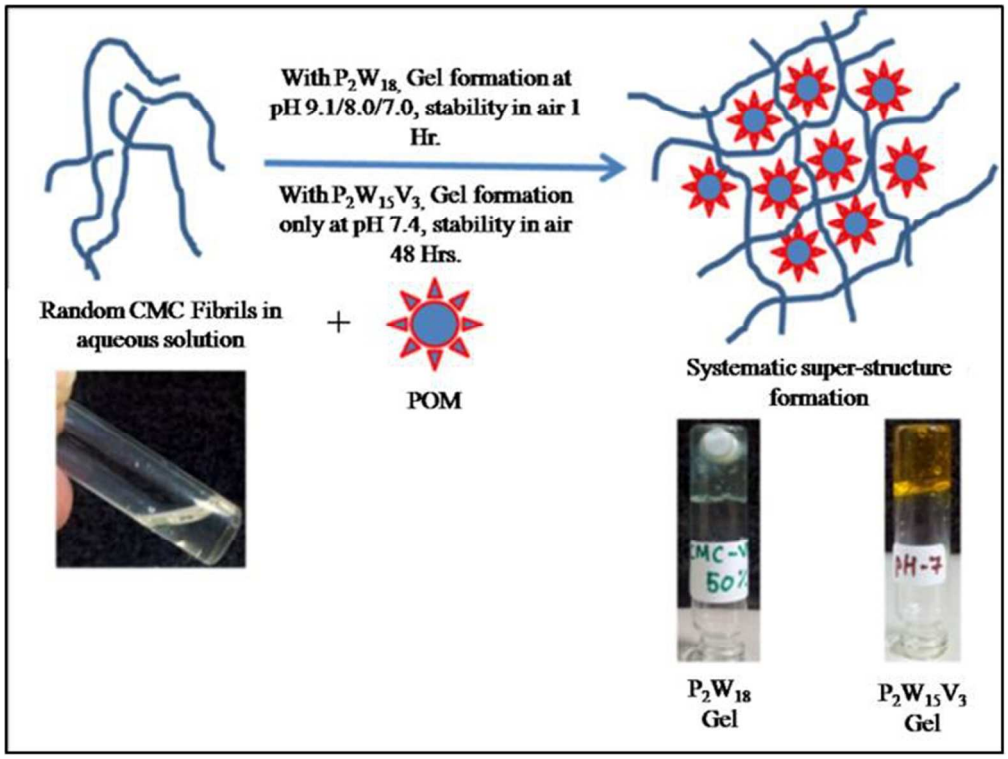
23 X. Wang, F. Li, S. Liu and M. T. Pope, *Journal of Inorganic Biochemistry*, 2005, **99**, 452.

24 Y. Yang, J. He, X. Wang, B. Li and J. Liu, *Trans. Metal chemistry*, 2004, **29**, 96.

25 M. T. Pope, Heteropoly and Isopoly Oxometalates, *Berlin: Springer-Verlag*, 1983**.**

26 C. R. Graham and R. G. Finke, *Inorganic Chemistry*, 2008, **47**, 3678

27 R. G. Finke, B. Rapko, R. J. Saxton and P. J. Domaille, J. *Am. Chem. Soc.* 1986, **108**, 2947.



Polyoxometalate assisted, pH dependent self-assembled hydrogel formation.
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