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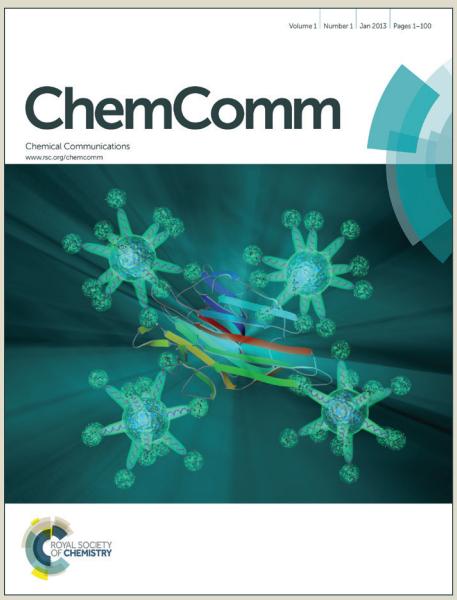

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COMMUNICATION

A Supramolecular Hydrogel Based on Carbamazepine†

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Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

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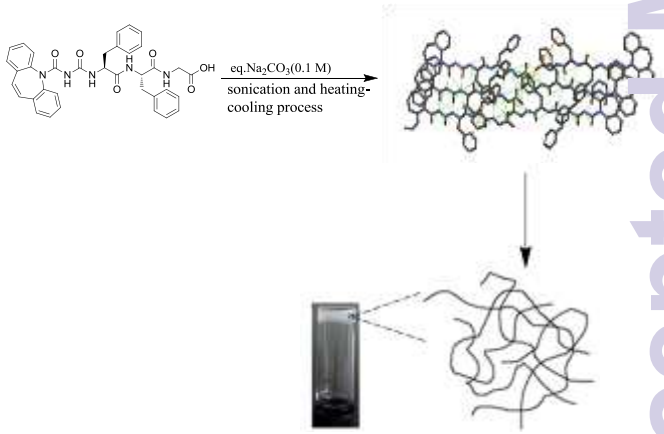
In this communication we report the first supramolecular hydrogel based on an antiepileptic drug carbamazepine (CBZ). CBZ plays the dual role of drug molecule and aromatic capping group in this self-delivery system.

Supramolecular hydrogel formed by self-assembly of small molecules is an emerging and appealing research area in the field of drug delivery.[1-4] It is widely used to enhance hydrophobic drugs' aqueous solubility[5-6] and to achieve sustained release profile of drug[5, 7-8]. Moreover, hydrogel is also used to obtain injection administration on account of its inherent thixotropy and reversibility.[5-6, 9-11] Peptide-based supramolecular hydrogel has been extensively explored as it is easier to modify and more susceptible to degradation compared with other hydrogels.[1, 12-13] In the process of self-assembly, aromatic capping groups play a pivotal role for their strong tendency to form aromatic stackings[14-17]. In previous researches, many excellent aromatic capping groups were found, such as fluorene (Fmoc),[18-19] naphthalene (Nap),[20-22] phenothiazine (PTZ)[23] derivatives. However, as foreign substances without treatment effect, these capping groups may cause different degrees of side effect. Therefore, finding a capping group derivated from a drug has great significance.

Carbamazepine is one of widely used antiepileptic drugs (AEDs), especially for the treatment of partial and generalized tonic-clonic epileptic seizures. Unfortunately, CBZ has poor aqueous solubility (120 μg/mL, 25 ℃), which leads to incomplete bioavailability and hampers parenteral administration of the drug. In addition, due to its narrow therapeutic window (4-12 μg/mL), regular blood concentration monitoring is required for clinical application.[24-26] In terms of CBZ's poly aromatic ring structure, we speculate that it is a useful capping group to facilitate the gelation of peptide-based gelator. Therefore, in this article, we intend to take the advantages of hydrogel to increase solubility of CBZ, obtain sustained release profile and realize other administration routes of the drug. Based on the above elucidation, we designed and synthesized a drug-peptide-based supramolecular gelator (CBZ-FFG) via covalently connecting CBZ to a short peptide (FFG), and we expected that it would eventually generate a gel (Scheme 1).



Scheme 1. Schematic illustration of the formation of gel.

After successfully synthesized the compound, we tested its gelling ability. A stable opaque gel was formed by sonication and heating-cooling process with minimum gelation concentration (MGC) of about 1.5 wt%, confirmed by vial inversion. The microscopic structure of the gel was examined by transmission electron microscope (TEM) and scanning electron microscope (SEM). As shown in Fig. 1, nanoribbons are present with the widths in the range of 18-50 nm and several micrometers in length. The local amplified image (inset of Fig. 1) reveals that nanofibrils' densely alignment builds up the nanoribbons. Its corresponding SEM image shows the growth of nanofibers, consistent with the results of TEM. Th

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†Electronic Supplementary Information (ESI) available: Details of materials, synthesis, characterization and measurements. See DOI: 10.1039/x0xx00000x

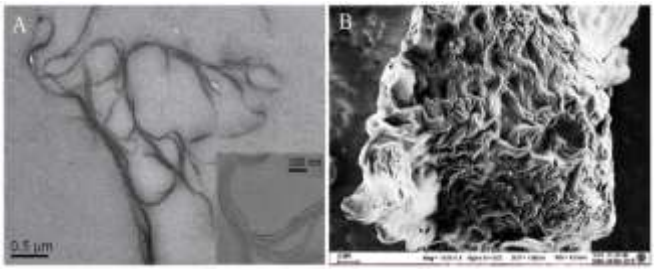

Fig. 1 TEM image (A) and SEM image (B) of the formed gel. The inset photo is the local amplified image.

gel without disturbance is stable at room temperature for several months by visual observation and it is furtherly observed by TEM. As shown in Fig. S-3A, the nanofibers aggregate into bundles after 3 months storage and the average width of the fibers is measured to be 30 nm. Moreover, we display the TEM image of the gel after releasing drug for 5 days, which also offers us an opportunity to catch a glimpse of the internal structure of the gel. As shown in Fig. S-3B, the long nanostructures degrade into short fragments after treatment with PBS buffer. This degradation confirms that the chemical bond has been broken up although the hydrogel matrix remains stiff.

Infrared spectroscopy (IR), fluorescence spectroscopy and circular dichroism (CD) were employed to understand the internal molecular arrangement in the formed gel. IR is a powerful tool to confirm the presence of hydrogen bonding. IR spectrum of the xerogel (Fig. S-4) shows a strong C=O stretching peak at 1670 cm^{-1} while the peaks at 3414 cm^{-1} and 1542 $cm^{-1}$are due to the N-H stretching vibration and N-H bending vibration, respectively. Compared with the IR spectrum of CBZ-FFG, a slight red-shift for C=O stretching peak indicates hydrogen bonding formation. A fluorescence emission experiment was performed to investigate the aromatic π–π stacking in the formed gel. As shown in Fig. S-5, the gelator solution exhibits a peak centered at 393 nm and the peak is shifted to 434 nm in the gel. This shift suggests the existence of π–π stacking in the gel.[27-30] The negative band at 197 nm in the CD spectrum (Fig. 2A) suggests that random coil nanostructures are largely existent in the formed gel. According to the above spectroscopic characterization, we can conclude that the formation of the gel is driven by the cooperative interactions of weak non-covalent interactions such as hydrogen bonds, π–π interactions, hydrophobic forces and nonspecific Van der Waals forces. Herein, we display a schematic illustration of the internal self-assembly of the gelator (Fig. S-6).

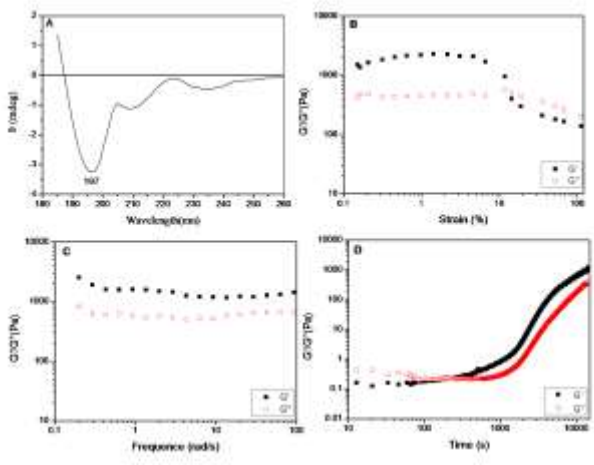

Fig. 2 CD spectrum of the gel (A, 2.0 wt%). Dynamic strain sweep of the gel (B, 2.0 wt%). Dynamic frequency sweep of the gel (C, 2 wt%). Dynamic time sweep of the gel (D, 2.0 wt%). The experiment was performed at 37 ℃.

Mechanical properties of the gel were measured by rheology study. According to the dynamic strain sweep (Fig. 2B), the values of the storage modulus (G′) dominate the loss modulus (G″) and the critical strain value of the gel is high to 6.56%, indicating a relatively strong gel formation. The gel exhibits a weak dependence on the frequency in the region of 0.1-100 rad/s in dynamic frequency sweep (Fig. 2C). Furthermore, the dynamic time sweep mode was carried out to characterize its reversibility. Before the measurement, we transformed the gel into its solution state by mechanical stirring indicated by the higher values of G″ than the values of G′ at the beginning in Fig. 2D. Then the gel displays a slow recovery with G′ becoming higher than G″. These results demonstrate good thixotropy and reversibility of the gel, thus making it a promising candidate for injectable therapies.

The release profile of CBZ from the gel at physiological temperature and physiological pH *in vitro* was studied. We used a LCMS-20AD to quantify the free CBZ. As shown in Fig. 3, the gel exhibits a two stage release profile of CBZ. It releases CBZ at a rate of about 2.06 μg per hour for the first 5 hours and then at a rate of about 0.22 μg per hour from the 5th to the 24th hour during experiment period. The accumulating release percentage of CBZ from gel in the 24 h period is about 1.04%. The release rate of drug from a hydrogel carrier involves two decisive processes. One is the regulated gel-sol transition which governs the leakage or diffusion of the drug.

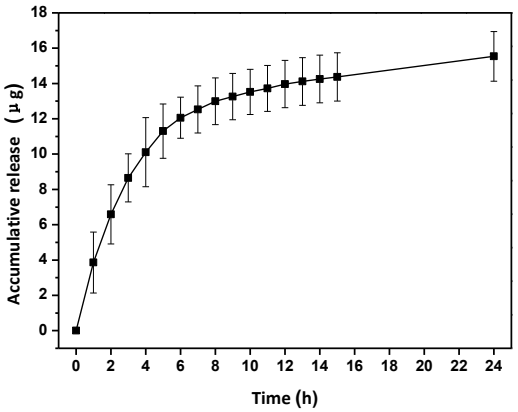

Fig. 3 *In vitro* release profile of CBZ from gel (37 ℃, pH=7.4, n=3).

The other is the chemical bond breaking process which is required for a self-delivery hydrogel to generate the original drug.[13, 31-32] The result shows faster release of CBZ in the first 5 hours. It is reasonable that the drug molecules at the interface between PBS solution and gel can be quickly released into the PBS buffer since the chemical bond degradation is the mainly rate-limiting step. We also observed that the gel was still rigid after treated with PBS buffer for 10 days. This indicats the potent sustained release of the drug from the gel.

In this paper, the biocompatibility of the hydrogel was studied at the same time. As shown in Fig. S-10, after being incubated with the gelators at 500 μM for 24 h, almost 96% cells remain alive through the MTT assay. The result exhibits the excellent biocompatibility of the gel and implies its potent application in drug delivery system.

To the best of our knowledge, among the reported drug-peptide-based supramolecular hydrogels, researches usually focused on anticancer[6, 33-36], anti-inflammatory[37-40], antibacteria[41] and antiviral[42] drugs. In this article we demonstrate the first example of a drug-peptide-based supramolecular hydrogel from an antiepileptic drug. This self-delivery system with high water solubility and sustained release profile will have an excellent prospect since it will reduce the necessary dosage and frequency of dosing and probably reduce undesired side effect of CBZ. It can be served as a subcutaneous implant or intravenous injection dispersion. Moreover, as a new capping agent based gel, it can be used to encapsulate other drugs to achieve synergistic therapy of epilepsy. However, more effort is needed to make this kind of hydrogel into practical application, for instance, more detailed *in vitro* and *in vivo* experiments.

The authors gratefully acknowledge the financial support from the National Natural Science Foundation of China (No. 81173023) and Priority Academic Program Development of Jiangsu Higher Education Institutions (PAPD).

Notes and references

1. G. Fichman and E. Gazit, *Acta Biomaterialia*, 2014, **10**, 1671-1682.
2. E. K. Johnson, D. J. Adams and P. J. Cameron, *Journal of Materials Chemistry*, 2011, **21**, 2024-2027.
3. K. J. Skilling, F. Citossi, T. D. Bradshaw, M. Ashford, B. Kellam and M. Marlow, *Soft Matter*, 2014, **10**, 237-256.
4. R. Lin and H. Cui, *Current Opinion in Chemical Engineering*, 2015, **7**, 75-83.
5. A. Altunbas, S. J. Lee, S. A. Rajasekaran, J. P. Schneider and D. J. Pochan, *Biomaterials*, 2011, **32**, 5906-5914.
6. C. Yang, Z. Wang, C. Ou, M. Chen, L. Wang and Z. Yang, *Chemical Communications*, 2014, **50**, 9413-9415.
7. M.-L. Briuglia, A. J. Urquhart and D. A. Lamprou, *International Journal of Pharmaceutics*, 2014, **474**, 103-111.
8. M. Singh, S. Kundu, A. M. Reddy, V. Sreekanth, R. K. Motiani, S. Sengupta, A. Srivastava and A. Bajaj, *Nanoscale*, 2014, **6**, 12849-12855.
9. A. Baral, S. Roy, A. Dehsorkhi, I. W. Hamley, S. Mohapatra, S. Ghosh and A. Banerjee, *Langmuir*, 2014, **30**, 929-936.
10. M. Guvendiren, H. D. Lu and J. A. Burdick, *Soft Matter*, 2012, **8**, 260-272.
11. L. Yu and J. Ding, *Chemical Society Reviews*, 2008, **37**, 1473-1481.
12. R. Li, C. Shu, W. Wang, X. Wang, H. Li, D. Xu and W. Zhong, *Journal of Pharmaceutical Sciences*, 2015, n/a-n/a.
13. C. Shu, R. Li, Y. Yin, D. Yin, Y. Gu, L. Ding and W. Zhong, *Chemical Communications*, 2014, **50**, 15423-15426.
14. S. Fleming and R. V. Ulijn, *Chemical Society Reviews*, 2014, **43**, 8150-8177.
15. D. M. Ryan, T. M. Doran and B. L. Nilsson, *Langmuir*, 2011, **27**, 11145-11156.
16. J. Shi, Y. Gao, Z. Yang and B. Xu, *Beilstein Journal of Organic Chemistry*, 2011, **7**, 167-172.
17. J. Zhou, X. Du, Y. Gao, J. Shi and B. Xu, *Journal of the American Chemical Society*, 2014, **136**, 2970-2973.
18. S. Fleming, S. Debnath, P. W. J. M. Frederix, T. Tuttle and R. V. Ulijn, *Chemical Communications*, 2013, **49**, 10587-10589.
19. R. V. Ulijn and A. M. Smith, *Chemical Society Reviews*, 2008, **37**, 664-675.
20. L. Chen, K. Morris, A. Laybourn, D. Elias, M. R. Hicks, A. Rodger, L. Serpell and D. J. Adams, *Langmuir*, 2010, **26**, 5232-5242.
21. L. Chen, S. Revel, K. Morris, L. C. Serpell and D. J. Adams, *Langmuir*, 2010, **26**, 13466-13471.
22. Z. Yang, G. Liang, M. Ma, Y. Gao and B. Xu, *Journal of Materials Chemistry*, 2007, **17**, 850-854.
23. C. Ou, J. Zhang, X. Zhang, Z. Yang and M. Chen, *Chemical Communications*, 2013, **49**, 1853-1855.
24. V. Franco, A. Iudice, E. Grillo, R. Citraro, G. De Sarro and E. Russo, *Expert Opinion on Pharmacotherapy*, 2014, **15**, 637-644.
25. K. Vucicevic, B. Milijkovic, R. Velickovic, M. Pokrajac, A. Mrhar and I. Grabnar, *Therapeutic Drug Monitoring*, 2007, **29**, 781-788.
26. F. Flicker, V. A. Eberle and G. Betz, *International Journal of Pharmaceutics*, 2011, **410**, 99-106.
27. M. Ma, Y. Kuang, Y. Gao, Y. Zhang, P. Gao and B. Xu, *Journal of the American Chemical Society*, 2010, **132**, 2719-2728.
28. M. J. Krysmann, V. Castelletto and I. W. Hamley, *Soft Matter*, 2007, **3**, 1401-1406.
29. A. M. Smith, R. J. Williams, C. Tang, P. Coppo, R. F. Collins, M. L. Turner, A. Saiani and R. V. Ulijn, *Advanced Materials*, 2008, **20**, 37-+.
30. D. K. Maiti and A. Banerjee, *Chemistry-an Asian Journal*, 2013, **8**, 113-120.
31. Y. Feng, M. Lee, M. Taraban and Y. B. Yu, *Chemical Communications*, 2011, **47**, 10455-10457.

32. E. A. Appel, R. A. Forster, M. J. Rowland and O. A. Scherman, *Biomaterials*, 2014, **35**, 9897-9903.
33. L. Mao, H. Wang, M. Tan, L. Ou, D. Kong and Z. Yang, *Chemical Communications*, 2012, **48**, 395-397.
34. Z. Song, H. Liu, J. Shen and X. Chen, *Biomaterials Science*, 2013, **1**, 190-193.
35. H. Wang, L. Lv, G. Xu, C. Yang, J. Sun and Z. Yang, *Journal of Materials Chemistry*, 2012, **22**, 16933-16938.
36. C. Yang, D. Li, Q. FengZhao, L. Wang, L. Wang and Z. Yang, *Organic & Biomolecular Chemistry*, 2013, **11**, 6946-6951.
37. J. Li, Y. Kuang, J. Shi, Y. Gao, J. Zhou and B. Xu, *Beilstein Journal of Organic Chemistry*, 2013, **9**, 908-917.
38. J. Majumder, M. R. Das, J. Deb, S. S. Jana and P. Dastidar, *Langmuir*, 2013, **29**, 10254-10263.
39. J. Majumder, P. Yedoti and P. Dastidar, *Organic & Biomolecular Chemistry*, 2015, **13**, 2300-2309.
40. R. Roy, J. Deb, S. S. Jana and P. Dastidar, *Chemistry – An Asian Journal*, 2014, **9**, 3196-3206.
41. Z. Yang, Y. Kuang, X. Li, N. Zhou, Y. Zhang and B. Xu, *Chemical Communications*, 2012, **48**, 9257-9259.
42. J. Li, X. Li, Y. Kuang, Y. Gao, X. Du, J. Shi and B. Xu, *Advanced Healthcare Materials*, 2013, **2**, 1586-1590.

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