Organic & Biomolecular Chemistry

Accepted Manuscript

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/obc

Journal Name

RSCPublishing

COMMUNICATION

Preparation of polydopamine nanocapsules in miscible tetrahydrofuran/buffer mixture

Cite this: DOI: 10.1039/x0xx00000x

Yun-Zhou Ni,[a] Wen-Feng Jiang,[a] Gang-Sheng Tong,[b] Jian-Xin Chen,[a] Jie Wang,[a] Hui-Mei Li,[a] Chun-Yang Yu,[a] Xiao-hua Huang,[c*] and Yong-Feng Zhou[a*]

Received 00th January 2012,
Accepted 00th January 2012

DOI: 10.1039/x0xx00000x

www.rsc.org/

Abstract

A miscible tetrahydrofuran/ tris **buffer mixture has been used to fabricate polydopamine hollow capsules with a size of 200 nm and with a shell thickness of 40 nm. An unusual non-emulsion soft template mechanism has been disclosed to explain the formation of capsules. The results indicate that the capsule structure is highly dependent on the volume fraction of tetrahydrofuran as well as the solvent, and the shell thickness of capsules can be controlled by the reaction time and dopamine concentrations.**

Hollow polymeric capsules, especially hollow polymeric nanocapsules have attracted considerable interest in recent years because of their wide application as drug delivery vectors, biomimetic reactors, sensors, and diagnostics.[1-4] As allowing the size, composition, morphology of the capsules to be finely tuned, template-assisted approach has widely used to prepare polymeric capsules.[5-7] Typically, hard[8-10] or soft[11] templates are used as sacrificial cores, and polymer can be deposited on the cores via some methods such as layer-by-layer (LbL) assembly[12, 13] or in situ polymerization[14]. Hard templates hold advantages of their stability and monodispersity, but harsh chemical reagents (e.g., acids, organic solvents) are usually required to dissolve these templates, which might bring about some limitations on the applications, especially biomedical applications. As a contrast, soft templates, generally based on emulsion systems, can be easily removed in mild conditions like aqueous alcohol solution. Due to this advantage, in past decades, various emulsion system have been developed to prepare polymeric capsules, such as hexadecane-in-water[15, 16], toluene-in-water[17], and isooctane-in-water[18]. There are two basic requirements to form these emulsion systems. One is that the emulsion needs at least two immiscible solvents, and the other is that surfactants are needed to stabilize emulsion. It is a big problem to remove the surfactants for the finally prepared capsules. So, it is useful and important to develop simpler and surfactant-free soft templates to fabricate hollow nanocapsules.

Polydopamine (PDA), inspired by mussel adhesive protein, has displayed excellent adhesive property on different substrates in alkaline solution, and exhibited good biocompatibility.[19-22] It has also been used to prepare hollow capsules via hard and soft template approaches. Caruso et al[20] applied SiO_2 particles as hard templates to fabricate hollow PDA capsules with a range of sizes and mesoporous structures. $CaCO_3$ spheres were also used to prepare PDA capsules in order to construct multienzyme system.[21] Recently, emulsion method (soft template approach) has been investigated to prepare PDA capsule. Caruso and Hao et al[22] successfully obtained monodisperse PDA capsules via using dimethyldiethoxysilane (DMDES)-in-Tris buffer system, and the size of capsules could be controlled from 400 nm to 2.4 μm, which was the earliest report involving soft template routes to fabricate PDA capsules. Wang and coworkers[23] employed pristine oil-in-water emulsions technology to prepare PDA capsule with sizes of 1.3−7.5 μm. Rahimipour and co-workers[24] combined soft emulsion (either canola oil or *n*-dodecane in Tris buffer) technique with sonochemical method and obtained PDA capsules with the size of 227 nm around. In general, the progress in the preparation of PDA capsules is still limited, especially in the preparation of smaller capsules below 300 nm.

In the present work, we report a new soft template method to prepare PDA nanocapsules around 200 nm. It is well-known that tetrahydrofuran (THF) is macroscopically miscible with water, and it is impossible for them to form emulsion. However, to our great surprise, PDA hollow nanocapsules were formed when we mixed dopamine with THF/Tris buffer mixtures at proper volume fraction of THF (φ). Mechanism study indicates THF/Tris buffer mixtures can form microscopically inhomogeneous domains which template the PDA capsules. So, our present work has extended the soft templates from emulsion to non-emulsion systems. In addition, the thickness of capsule membrane can be controlled by dopamine concentration, and is highly dependent on the reaction time. Furthermore, in principle, any kind of agents that are soluble in THF, can be easily encapsulated in the capsules after PDA self-polymerization on the THF/water droplet interface, offering potential applications in biotechnology, drug delivery systems and devices.[22] All these characteristics in combination with the advantages of facile preparation, small capsule size, no surfactants and easy template-removing process by evaporation, make this non-emulsion method very promising in the preparation of polymeric capsules.

The synthesis process is very simple just by adding dopamine into the THF/Tris buffer mixtures with a typical concentration of 0.5

mg/ml. The reaction process of dopamine at room temperature (25 °C) was monitored by UV-vis spectrometer. As shown in Figure 1a (φ=0.7), two new absorption peaks around 300 nm and in the range of 400-700 nm appear with the reaction, respectively. The former peak is assigned to the oxidation reaction of dopamine into dopachrome; the later one is contributed to the continuous self-polymerization of dopamine into PDA.[25] We thus measured the absorption at λ=400 nm with reaction time to evaluate the self-polymerization dynamics.[23] As shown in Figure 1b, the absorption increases sharply after 100 hrs and then levels off after 168 h, meanwhile the reaction system changes from colorless to dark. All these results clearly support the successful self-polymerization of PDA during our experimental condition. A proposed PDA self-polymerization mechanism including covalent polymerization and non-covalent self-assembly processes has been sumarized in the supporting information (Figure S1, ESI†).

The particles obtained from the reaction system were then characterized by TEM (Figure 1c), which shows the particles have a clear contrast difference between the inner pool and the outer black wall, indicating the formation of PDA hollow capsules. The capsules are around 200 nm in diameter and are flexible to be deformed during the TEM sampling process. SEM (Figure 1d) was also used to characterize the capsules and the hollow structure was directly seen according to the cavities of the particles (red arrows). PDA capsules could also be obtained when more THF was added in the reaction systems (φ=0.8) according to the TEM (Figure 1e) and AFM (Figure 1f) measurements. The AFM image clearly shows the capsule structure with a hole in a three-dimensional mode (inset of Figure 1f). A higher temperature of 45 °C was used in order to increase the solubility of dopamine at φ=0.8.

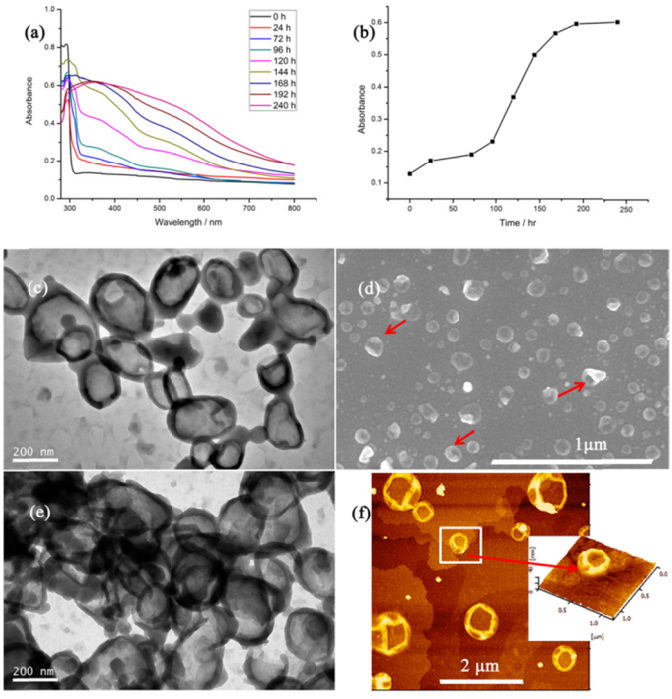

Figure 1. (a) UV-vis spectra of dopamine polymerization in THF/tris buffer (φ=0.7) mixture. (b) Time evolution of the absorbance of dopamine self-polymerization in mixture at 400nm. The TEM (c) and SEM (d) images of PDA capsules at φ=0.7. The TEM (e) and AFM (f) images of PDA capsules reacted at φ=0.8 and 45 °C.

The data in figure 1 prove that PDA nanocapsules have been prepared through the self-polymerization of dopamine in thre miscible THF/Tris buffer mixtures. As mentioned above, PDA capsules are generally prepared through an immiscible emulsion template, so how can the capsules be formed in THF/Tris buffer non-emulsion system? To address this, the intermediates with different reaction time were collected for TEM observation. Figure 2a shows the TEM image of the samples after reacted for 12 hrs. Many discrete small PDA nanoparticles together with some big hollow spheres are observed, and the hollow spheres are coated with PDA nanoparticles according to the magnified image (inset). Wu et al.[26] had studied solution dynamics of THF/water mixture by using laser light scattering technology and found that the mixture was not microscopically homogeneous and could form complexes with a dynamic correlation length of 200-600 nm. Our results presented here agree well with Wu's work. It was the microphase-separated THF/water complexes that template the adsorption and self-polymerization of dopamine on the surface, which lead to the final formation of PDA hollow spheres. As reported by Wu, the THF/water complexes are not stable, however, in our system, PDA nanoparticles spontaneous aggregate onto the surface of the complexes and will probably stabilize them through a "Pickering emulsion" mechanism (inset of Figure 2a).[27, 28]

After reacted for 36 hrs, hollow spheres with much clearer rim were observed (Figure 2b), and PDA nanoparticles began to merge together on the surface of the hollow spheres to form the thicker shell according to the amplified image (inset of figure 2b). After reacted for 72 hrs, many hollow spheres with clear shells were obtained (Figure 2c). Figure 2d shows the changes of the shell of the PDA capsules with reaction time. The shell thickness progressively increased from about 5nm, 7 nm, 13 nm to 31 nm after reacted for 12 hrs, 36 hrs, 72 hrs, and 240 hrs, respectively. A similar result was obtained when we set the reaction time at 192 hrs and φ=0.7 but changed the dopamine concentrations. The capsule shell thickness increased from 7nm, 29 nm to 40nm when the dopamine concentration increased from 0.1 mg/ml, 0.5 mg/ml to1.0 mg/ml (Figure S2, ESI†), respectively. So, the capsule shell thickness can be controlled by adjusting either the reaction time or dopamine concentration. The shell thickness of polymeric capsules plays a crucial role in the permeability and the mechanical strength of the shell wall, which is an important factor for the applications.[29,30]

The abovementioned results strongly support a template-triggered growth mechanism as shown in figure 3 for the formation of PDA nanocapsules. The mixture of THF/buffer is macroscopically homogeneous; however, microphase-separated THF/buffer nanodrops aroud 200-600 nm will be formed in microscopic level. These nanodrops serve as the templates to accommodate the self-polymerization of dopamine on the surface. With the continuous accumulation and growth of PDA layers on the surface of the templates, PDA capsules with thick shells are obtained, and the shell thickness increases with the reaction time. The same shell thickness and reaction time dependence has been found in the silica particle-templated PDA capsule system.[20]

Additionally, the stability of PDA capsules in THF/Tris buffer mixture (φ=0.7) was examined. The mixture was kept for 5 months at 4 °C. It was found that plenty of PDA nanocapsules still existed in the mixture according to SEM measurements (Figure S3, ESI†), indicating the stability of the PDA nanocapsules. In fact, the PDA are crosslinked each other in the nanocapsules, so they are quite stable. Nevertheless, it should be noted that the capsules are more crimpled or deformed than the freshly prepared ones, and some of them tend to aggregate together after 5 month's storage.

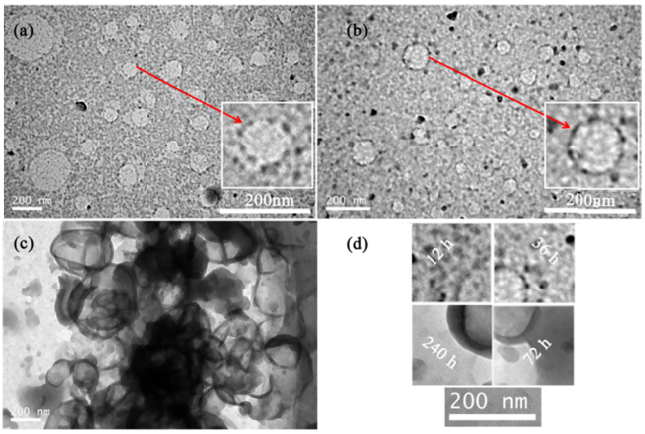

Figure 2 The TEM images of PDA capsules at φ=0.7 and 25 °C with different reaction time of 12 hrs (a), 36hrs (b), and 72hrs (c). (d) The TEM images of PDA capsule shells when reacted for 12 hrs, 36 hrs, 72 hrs, and 240 hrs, respectively. The sample solutions reacted at 12 hrs (a), 36hrs (b), and 72hrs (c) were directly used for TEM observation without any purification.

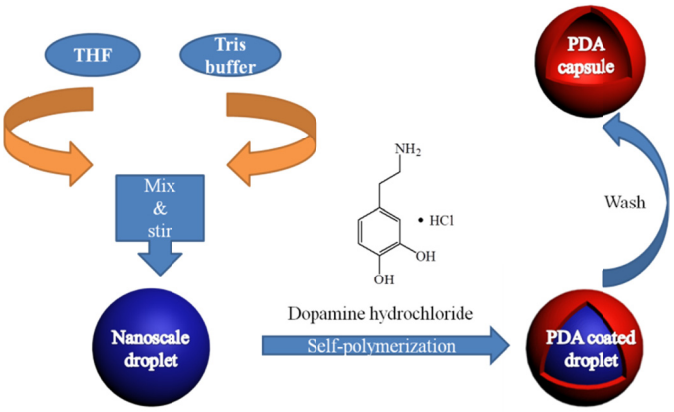

Figure 3. Preparation procedure of the PDA hollow capsules via THF/Tris buffer mixtures.

The effect of the volume fraction of THF (φ) on the PDA nanocapsules was further studied. For this purpose, the dopamine polymerization in THF/Tris buffer mixture with different φ was carried out. The time for the accomplishment of dopamine polymerization in the mixture (φ=0, φ=0.2, φ=0.5) was evaluated by the absorption intensity at 400nm with reaction time (Figure S4, ESI†), and they were around 4 h, 22 h, 67 h respectively. It indicates that the self-polymerization rate of dopamine decreases with the increase of the volume fraction of THF (φ). This may be attributed to the low concentration of dissolved oxygen in the system with the increase of THF content, which impedes the oxidation of dopamine and its self-polymerization.

The self-assembled morphology is also dependent on the volume fraction of THF (φ). Figures 4a-b shows the TEM & SEM images of the PDA particles obtained in 100% Tris buffer (φ=0), which indicates the formation of PDA solid spheres with the size of about 200-300nm as usual. When φ=0.1, similar to the pure Tris buffer, only PDA solid particles with the sizes around 200 nm were formed (Figures 4c and d). When φ=0.2, PDA particles with the size mainly around 200-300 nm were also formed (Figures 4e and f). However, hollow lumens (red arrows, Figure 4e) around 100-200nm were observed in some of these particles, and the thickness of shell was about 100 nm. When φ=0.3, the hollow spheres around 200 nm and with the shell thickness of 40nm could be widely observed (Figures 4g and 4h). So, only when the THF content is above a critical fraction (φ=0.2) will the microphase-separated THF/water nanodrops act as soft templates to template the PDA hollow capsules. Wu and coworkers[26] also found the formation of THF/water complex around 200 nm when the THF molar fraction was larger than 0.13. So our results agree well with their results, which is a further evidence to support the mechanism as shown in figure 3. The shell thickness of PDA capsules becomes thinner from φ=0.2 to φ=0.3, which is probably due to the increase of concentration of the nanodroplet templates with the increase of THF volume fraction. As a result, less PDA polymers are coated onto the templates, leading to thinner capsule shells. It should be noted that there are no clear morphology difference between the PDA particles at different THF content when measured by SEM, and only spherical particles are observed.

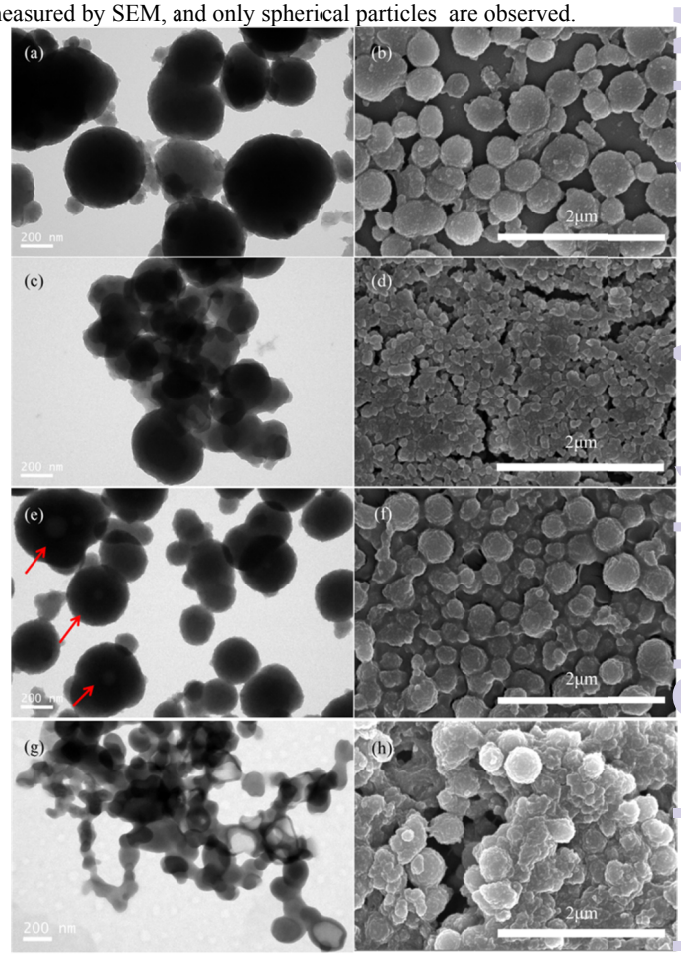

Figure 4. The TEM and SEM images of PDA particles obtained via 10 days oxidation and self-polymerization of dopamine in THF/Tris buffer mixtures at φ=0 (a, b), φ=0.1 (c,d), φ=0.2 (e,f) and φ=0.3 (g,h). The samples were purified by centrifugation before measurements.

Besides, the effects of buffer and solvent on the formation of PDA nanocapsules are also studied. In the experiments, DA was added into THF/Phosphate buffer mixture or Dimethyl Sulfoxide (DMSO)/Tris buffer mixture with a DA concentration of 0.5mg/ml and φ=0.7. The pH value of each buffer was set at 8.0, and the reactions were carried out at 25 °C via 7 days (168 h). It is found PDA nanocapsules around 200 nm can also be obtained in THF/Phosphate buffer mixture (Figure S5, ESI†). For DMSO/Tris buffer mixture reaction system, only very small PDA nanoparticles with the size around 2 nm instead of PDA nanocapsules were found (Figure

S6, ESI†). In addition, as reported by Lee and coworkers[31], only PDA solid spheres with tunable diameters from 70 nm to 300 nm were obtained in alcohol/Tris buffer mixture. These results partially support that the preparation of PDA capsules is not related with the buffer solution, but is highly related with the solvent. DMSO or alcohol is highly soluble in water, so both of them are unable to form the microphase-separated microdomains as the soft templates in DMSO/water and alcohol/water mixtures to medicate the formation of nanocapsules.

Conclusions

In summary, we demonstrates here a simple and non-emulsion method by using THF and buffer miscible mixture to fabricate polydopamine hollow capsules around 200 nm in size. Mechanism study indicates THF/buffer mixture can form some microphase-separated complexes to template the formation of capsules. The capsule shell thickness can be controlled by adjusting reaction time and dopamine concentration, and increases with increasing the reaction time and dopamine concentration. The formation of polydopamine capsules is also highly dependent on the volume fraction of THF as well as the water miscible solvent, and the capsules can only be formed tile the THF fraction is larger than 0.2, and can not be formed in DMSO/buffer and alcohol/buffer mixtures. This work has shown the great possibility to produce hollow nanostructures by using macroscopically miscible but not microscopically homogeneous mixed solvent as soft templates.

Experimental Section

Materials TRIS buffer consisting of Disodium ethylenediaminetetraacetate dehydrate and Tris(hydroxymethyl) aminomethane was purchased from Aladdin Industrial Inc., Tetrahydrofuran (THF), Dimethyl Sulfoxide (DMSO), Phosphate buffer consisting of Sodium dihydrogen phosphate dehydrate and Disodium hydrogen phosphate dodecahydrate were purchased from Sinopharm Chemical Reagent Co., Ltd, 3-hydroxytyramine hydrochloride (dopamine) was purchased from Acros and used without purification. The water used in all experiments was prepared in Milli-Q purification system and had a resistivity greater than 18.2 MΩ cm.

Oxidation and Self-Polymerization of Dopamine in THF/Tris mixture Typically, THF and Tris buffer or Phosphate buffer with a desired volume ratio are separately added in a flask. Then, the top of the flask was screwed and the mixture was stirred when dopamine hydrochloride was added to the mixed solution. Unless otherwise specified, the pH value of every buffer is 8.0, the concentration of each buffer is 10 mM, and the reaction system was carried out at 25℃ with 0.5mg/mL (based on mixed solvent) via 10 day.

TEM Transmission electron microscopy (TEM) imaging was implemented using a Tecnai G2 spirit Biotwin (FEI, USA) with a Gatan 832 microscope operated at an acceleration voltage of 120 kV. Unless otherwise specified, the sample was prepared by dropping the reaction solution onto copper grids coated with a thin carbon film and dried for several minutes, and then the grids were washed with deionized water (2-3 drops) with the help of filter paper. The sample was dried at room temperature for 48 h before TEM measurements. No staining treatment was performed for the measurement.

SEM The Scanning electron microscopy (SEM) imaging were obtained using a JSM-7401F (JEOL Ltd., Japan) field emission scanning electron microscope operated at an acceleration voltage of 5 kV. Unless otherwise specified, the sample was prepared by dropping the reaction solution onto silica wafers and dried for several minutes, and then the grids were washed with deionized water (2-3 drops) with the help of filter paper. The sample was dried at room temperature for 48 h before SEM measurements. Then the samples were sputter coated with gold to minimize charging.

AFM The Atomic force microscopy (AFM) imaging were obtained using a Nanonavi E-Sweep (SII, Japan). The surface morphologies of samples were acquired in tapping mode. The samples were prepared by dropping the reaction solution onto mica sheet and dried for several minutes, and then the mica sheet were washed with deionized water (2-3 drops) with the help of filter paper. The sample was dried at room temperature for 48 h before measurements.

UV-vis The UV-vis absorption spectra were obtained with a UV/V-16/18 (Mapada, China). Samples of reaction system were added to a 1cm quartz cuvette for the measurements.

Acknowledgements

The authors thank the financial supports from the National Basic Research Program (2013CB834506), China National Funds for Distinguished Young Scholar (21225420), National Natural Science Foundation of China (91127047, 21474062), "Shu Guang" project supported by Shanghai Municipal Education commission and Shanghai Education Development Foundation (13SG14), and the fund of Guangxi Natural Science Foundation (No.2014GXNSFAA118040).

Notes and references

[a]*School of Chemistry and Chemical Engineering, State Key Laboratory of Metal Matrix Composites, Shanghai Jiao Tong University,800 Dongchuan Road, Shanghai 200240, P. R. China. E-mail: yfzhou@sjtu.edu.cn*

[b]*Instrumental Analysis Center, Shanghai Jiao Tong University, 800 Dongchuan Road, Shanghai 200240, P. R. China*

[c]*Key Laboratory of New Processing Technology for Nonferrous Metal & Materials, Ministry of Education, and School of Material Science and Engineering, Guilin University of Technology, Guilin 541004, P.R. China. E-mail: hxh2002405@163.com*

† Electronic Supplementary Information (ESI) available: PDA self-polymerization dynamics and concentration effect for the capsule formation. See DOI: 10.1039/c000000x/

1. J. Cui, M. P. van Koeverden, M. Müllner, K. Kempe and F. Caruso, *Adv. Coll. Int. Sci.*, 2014, 207, 14-31.
2. P. Tanner, P. Baumann, R. Enea, O. Onaca, C. Palivan and W. Meier, *Acc. Chem. Res.*, 2011, 44, 1039-1049.
3. S. F. M. van Dongen, H.-P. M. de Hoog, R. J. R. W. Peters, M. Nallani, R. J. M. Nolte and J. C. M. van Hest, *Chem. Rev.*, 2009, 109, 6212-6274.
4. D. Lensen, D. M. Vriezema and J. C. M. van Hest, *Macromol. Biosci.*, 2008, 8, 991-1005.
5. F. Caruso, *Chem. – Eur. J.*, 2000, 6, 413-419.
6. G. B. Sukhorukov, A. L. Rogach, B. Zebli, T. Liedl, A. G. Skirtach, K. Köhler, A. A. Antipov, N. Gaponik, A. S. Susha, M. Winterhalter and W. J. Parak, *Small*, 2005, 1, 194-200.
7. Y. Wang, A. S. Angelatos and F. Caruso, *Chem. Mater.*, 2007, 20, 848-858.
8. P. Schuetz and F. Caruso, *Adv. Funct. Mater.*, 2003, 13, 929-937.
9. Z. Shi, Y. Zhou and D. Yan, *Macromol. Rapid. Commun.*, 2006, 27, 1265-1270.
10. Z. Shi, Y. Zhou and D. Yan, *Polymer*, 2006, 47, 8073-8079.
11. E. Tjipto, K. D. Cadwell, J. F. Quinn, A. P. R. Johnston, N. L. Abbott and F. Caruso, *Nano Letters*, 2006, 6, 2243-2248.

12. F. Caruso, R. A. Caruso and H. Möhwald, *Science*, 1998, 282, 1111-1114.
13. C. Gao, E. Donath, S. Moya, V. Dudnik and H. Möhwald, *Eur. Phys. J. E.*, 2001, 5, 21-27.
14. L. S. Zha, Y. Zhang, W. L. Yang and S. K. Fu, *Adv. Mater.*, 2002, 14, 1090-1092.
15. F. Tiarks, K. Landfester and M. Antonietti, *Langmuir*, 2001, 17, 908-918.
16. W. Li, K. Matyjaszewski, K. Albrecht and M. Möller, *Macromolecules*, 2009, 42, 8228-8233.
17. L. Zhang, P. Liu, L. Ju, L. Wang and S. Zhao, *Macromol. Res.*, 2010, 18, 648-652.
18. J. Jang and K. Lee, *Chem. Commun.*, 2002, 1098-1099.
19. H. Lee, S. M. Dellatore, W. M. Miller and P. B. Messersmith, *Science*, 2007, 318, 426-430.
20. A. Postma, Y. Yan, Y. Wang, A. N. Zelikin, E. Tjipto and F. Caruso, *Chem. Mater.*, 2009, 21, 3042-3044.
21. L. Zhang, J. Shi, Z. Jiang, Y. Jiang, S. Qiao, J. Li, R. Wang, R. Meng, Y. Zhu and Y. Zheng, *Green. Chem.*, 2011, 13, 300-306.
22. J. Cui, Y. Wang, A. Postma, J. Hao, L. Hosta-Rigau and F. Caruso, *Adv. Funct. Mater.*, 2010, 20, 1625-1631.
23. H. Xu, X. Liu and D. Wang, *Chem. Mater.*, 2011, 23, 5105-5110.
24. G. Yeroslavsky, M. Richman, L.-o. Dawidowicz and S. Rahimipour, *Chem. Commun.*, 2013, 49, 5721-5723.
25. W. J. Barreto, S. Ponzoni and P. Sassi, *Spectrochim. Acta A: Mol. Biomol. Spectrosc.*, 1998, 55, 65-72.
26. C. Yang, W. Li and C. Wu, *J. Phys. Chem. B.*, 2004, 108, 11866-11870.
27. S. U. Pickering, *J. Chem. Soc., Trans.*, 1907, 91, 1988-2001.
28. J. Xu, A. Ma, T. Liu, C. Lu, D. Wang and H. Xu, *Chem. Commun.*, 2013, 49, 10871-10873.
29. X. Shi and F. Caruso, *Langmuir*, 2001, 17, 2036-2042.
30. A. A. Antipov, G. B. Sukhorukov, E. Donath and H. Möhwald, *J. Phys. Chem. B.*, 2001, 105, 2281-2284.
31. J. Yan , L. Yang , M. Lin , J. Ma , X. Lu , and P. Lee *Small* 2013, 9, 596–603