A new strategy for synthesis of porous magnetic supraparticles with excellent biodegradability†

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Porous magnetic supraparticles (p-MSPs) with surface area up to 285.4 m² g⁻¹ have been fabricated by a one-step etching method, which is 4 times greater than the unetched counterpart. They exhibit significantly better biodegradability than their counterpart in both mimicked physiological buffer solution and the cellular environment of HeLa cells.

Benefiting from extremely high porosity and the ability to tune pore size, porous nanomaterials including mesoporous titanium dioxide (m-TiO₂),1a mesoporous silica (m-SiO₂),1b,c porous carbon,1d covalent organic frameworks (COF)1e and metal–organic frameworks (MOF)1f have been exploited for various applications.1–3 These include serving as vehicles or reservoirs for drug delivery,3a,b as adsorbents3c–e and heterogeneous catalysts or catalyst supports.3f

Recently, we have pioneered a solvothermal-based method to synthesize sub-micron-sized mesoporous Fe₃O₄ colloidal nanocrystal clusters, named magnetic supraparticles (MSPs).4 During the synthesis procedure, polymer-stabilized magnetic supraparticles co-assemble with the polymer stabilized gas bubbles to form the nanocrystal clusters. The pores are in situ formed without the need for adding materials.4d Moreover, the MSPs that are composed of iron oxides can be degraded in acidic medium simulating the tumour cellular environment and endosome,4b,c as we previously reported.5 This MSP synthesis method can provide effective albeit limited controllability of pores. Furthermore enhancement of porosity and adjustment of pore size remain elusive.

Herein, we report a new strategy to generate porous MSPs (p-MSPs) by utilizing the etching properties of a DMF solution of methyl mercaptoacetate, which is a complexing agent and hydrazine, which is a reducing agent. The Fe₃O₄ component of MSPs can be reduced gradually by hydrazine to form Fe(OH)₂, which will be coordinated with methyl mercaptoacetate to dissolve in DMF. As a result, the interspaces will be increased as well as the porosity of the MSPs. The as-prepared p-MSPs possess an unusually larger pore volume and surface area than their counterpart without this additional etching step. They show a higher acidic degradation rate and have the potential to be applied in the delivery and storage of guest molecules.

As previously reported, the MSPs stabilized by agarose possess solid inner and roughened surfaces (Fig. 1a). However, when we added the MSPs into the DMF solution containing hydrazine, a reducing agent, and methyl mercaptoacetate, a chelating agent, to react at 80 °C, the structure of the MSPs clearly changed. After reacting for 30 min, some new interspaces appear in the MSPs (Fig. 1b). With a gradual increase of the reaction time, the interspaces in MSPs became more and

Fig. 1 The TEM images of (a) MSPs stabilized by agarose, the MSPs reacted with hydrazine and methyl mercaptoacetate in DMF for (b) 30 min, (c) 45 min, and (d) 60 min. All the bars are 100 nm.
more noticeable. This is as evidenced by greater numbers of tortuous channels that are generated in the MSPs after 60 min reaction (Fig. 1d). If the reaction time is allowed to continue, the skeleton of the MSPs is broken down after 120 min (Fig. S1 and S2, ESI†).

Table 1 shows the variation of surface area and pore volume with reaction time. At the beginning of the reaction, the surface area increased gradually, and reached the max value of 285.4 m² g⁻¹ after 60 min, which is significantly greater than that of the precursor MSPs (~58.5 m² g⁻¹).† This result indicates that the generated interspaces indeed could elevate the surface area of MSPs effectively. When the reaction time exceeded 60 min, the surface area of MSPs started to decrease gradually, and the pore volume of the p-MSPs decreased as well. This is because the skeleton started to collapse. Therefore, by controlling the reaction time, the property of p-MSPs including surface area can be adjusted accordingly.

In addition, with the increase of etching time, the magnetization value of p-MSPs is gradually decreased (Fig. S3, ESI†). This suggests either the reduction of the amount of magnetite in the particles or possible deterioration of the crystallinity of p-MSPs, which was detected by PXRD. The PXRD results showed that the crystallization of p-MSPs hardly changed prior to p-MSPs breaking down (Fig. S4a, ESI†). The TGA results (Fig. S4b, ESI†) depict that the percentage of the stabilizer in the whole particle increases along with etching time, implying that the amount of magnetite decreased with the etching time. Moreover, through analysis of the p-MSPs by FT-IR (Fig. S5, ESI†), we found that the ratio of the C-O peaks (ascribed to the carbohydrate chain of agarose)⁶c at ~1100 cm⁻¹ and the C=O (ascribed to the carboxyl group coordinating with Fe₃O₄)⁶c peak at ~1600 cm⁻¹ increased with a longer reaction time. This indicates that the content of Fe₃O₄ in p-MSPs decreased and thus the porosity of p-MSPs increased by etching of Fe₃O₄ in MSPs.

Analyzing the changes in the p-MSPs before and after etching, our hypothesis is that the generation of interspaces in p-MSPs may be ascribed to the loss of surface Fe₃O₄ due to the reduction of Fe(m) to Fe(u).⁷ Since hydrazine can reduce Fe₃O₄ to Fe(u) rapidly, and the product Fe(u) can be coordinated with methyl mercaptoacetate in DMF, Fe₃O₄ in MSPs will be etched.

We noticed that as the amount of complexing agent or reductant reduces to zero in the reaction system, the porous structure cannot be generated in the MSPs (Fig. S6 and S7, ESI†). Moreover, if the hydrazine was substituted with ethylenediamine, which is not a reducing agent but has two amine groups, the MSPs were not etched to p-MSPs as well (Fig. S8, ESI†). These two pieces of evidence show that the reduction from Fe(m) to Fe(u) by hydrazine is an important step.

We also found that some non-magnetic black powders were produced in addition to the p-MSPs. XPS (Fig. S9, ESI†) of the black powder showed that the Fe peaks are Fe(u) and the peaks of S are ascribed to the H–S bond and the Fe–S bond.⁶b,⁶a This shows that Fe(u) was indeed reduced and was coordinated with sulphydryl. Furthermore the PXRD data (Fig. S10, ESI†) of non-magnetic black powder indicate that the Fe(u) complex was amorphous. These observations led to the conclusion that the etching process is achieved by hydrazine together with methyl mercaptoacetate gradually peeling off the skin of Fe₃O₄ nanocrystals in MSPs to generate the void space.

One of the important applications of porous magnetic nanomaterials is drug delivery. We studied the degradation of p-MSPs in acidic medium. As shown in Fig. 2, with the increase of the etching time, the degradation rate of p-MSPs in pH = 5.0
buffer increased significantly as evidenced by the greater amount of Fe dissolved in pH 5 buffer solution for p-MSP samples etched for a longer time. Compared with the MSPs, the p-MSPs etched for 60 min showed a much faster degradation rate. The p-MSPs etched for 60 min could be degraded completely after 180 h of incubation whereas only 40 wt% was dissolved for MSPs. This result can be attributed to the combination of the faster diffusion rate of acid into the interspaces of p-MSPs, and the faster dissolution rates due to the active surface formed after hydrazine/methyl mercaptoacetate etching. Therefore, the formation of interspaces through etching will improve the acidic degradation of p-MSPs.

To further investigate the biodegradation behavior of p-MSPs, HeLa cells were employed as model cells. Our experiments showed that the p-MSPs are non-cytotoxic [Fig. S11, ESI†]. The selective etching method retained agarose in p-MSPs, the stabilizer, which promotes their biocompatibility. After staining with an Fe³⁺ sensitive probe, the distribution of Fe³⁺ (dissolved from p-MSPs) in HeLa cells can be observed using a confocal laser scanning microscope (CLSM). In Fig. 3, only small areas of red fluorescence were detected in the cells incubated with MSPs whereas strong red fluorescence was observed in the cells incubated with p-MSPs. The intensity of red fluorescence reflects the concentration of Fe³⁺ in the cells, indicating faster degradation of p-MSPs. This result demonstrated that the p-MSPs with high surface area exhibit enhanced biodegradation ability in HeLa cells, coinciding with simulated degradation experiment results in pH = 5.0 buffer. Thus, combining high surface area with good acidic degradability, p-MSPs offer great potential to serve as biodegradable drug carriers.

In conclusion, we have synthesized p-MSPs via a one-step etching method of MSPs. p-MSPs stabilized by agarose offer greatly higher porosity relative to MSPs. Furthermore, the surface area and pore volume can be readily adjusted by etching time. Owing to the extremely large surface area and activated surface, p-MSPs exhibit greatly enhanced acidic degradation capability in both buffer and the cellular environment. Since the retainable stabilizer, agarose, can be further modified to endow p-MSPs with more functionalities, we believe that this new system can enable a wide range of applications such as catalysis, separation, and drug delivery.

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Notes and references