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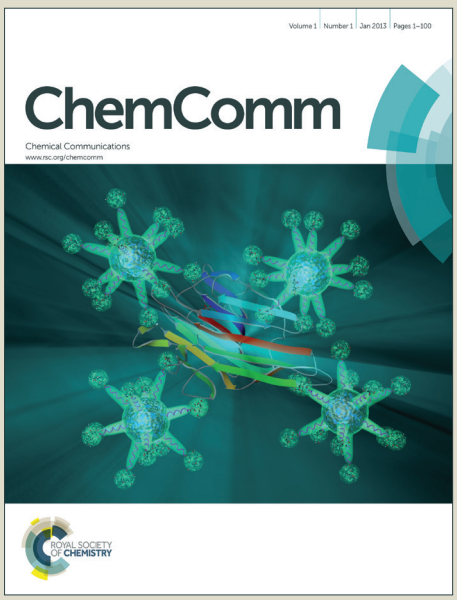

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Hierarchical porous polycaprolactone microspheres generated in a simple pathway combining nanoprecipitation and hydrolysis†

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We demonstrated a one-pot, soap-free fabrication of porous polycaprolactone microspheres by combining nanoprecipitation and hydrolysis. Obtained porous polycaprolactone microspheres show a great advantage in the application of drug delivery.

The morphology control of polymer microparticles has received great attention for decades due to its importance in potential applications.[1] Various polymer microparticles with peculiar morphologies have been generated through multistage polymerizations, double emulsion, interfacial interactions induced reconstruction, microfluidics, etc.[1d,2] In addition, the crystallization property of polymer has shown some influence on particle's morphology, leading to the formation of coral-like, plate-like, cone-like and sheaf-like particles.[3] Among these unique particles, porous particles, which have high specific surface area, have been utilized in numerous areas such as catalysis, tissue engineering, chromatography, and drug delivery.[4] Compared with the fabrication of solid polymer particles, constructing porous structure generally involves multiple and time-consuming procedures.[5]

Double emulsion has been considered to be an efficient method to produce porous microspheres, especially for biodegradable polymers applied in drug delivery and controlled release.[6] In general, double emulsion is formed through a two-step emulsification process using two kinds of surfactants, oil-soluble and water-soluble, to stabilize the o/w and w/o interfaces. However, double emulsion is an unstable system. The second emulsification step may introduce destabilization through rupture of the inner droplets. Recently, porous polymer microspheres fabricated by double emulsion have been prepared by one-step process using an unitary surfactant.[2d,6e,7] However, the residual surfactant in polymer particles may have toxicity that limits their application in biomedical area.[8] To solve this problem, amphiphilic biocompatible block copolymer was used as unitary surfactant to produce w/o/w emulsions, thus resulting in porous microparticles.[7b] In some special cases, porous microspheres can be easily prepared by one-step soap-free emulsion polymerization of amphiphilic block copolymer.[6e,9] Amphiphilic copolymer is considered as a crucial factor to create surfactant-free double emulsion.

Herein we demonstrate a unique strategy to fabricate porous polymer microspheres. We introduce hydrolysis into nanoprecipitation to generate amphiphilic polymer chains in situ in micro-droplets that play as surfactant to create double emulsion effect. Polycaprolactone (PCL) is chosen as model polymer to prove this concept. In this nanoprecipitation process, NaOH is used as nonsolvent, which will lead to the hydrolysis of PCL. As a consequence, the hydrolyzed PCL becomes more hydrophilic and works as a surfactant to stabilize oil-water interface inside droplets. Cage-like and plate-like PCL porous microspheres are generated in different experimental conditions. This result expands the application of nanoprecipitation to manipulating porous polymer microparticles for the first time. Our method combines the advantages of nanoprecipitation and double-emulsion method, showing a synergistic effect in a simple one-pot process. The obtained cage-like PCL porous microspheres were loaded with doxorubicin (DOX) through electrostatic interaction and checked its controlled-release in different pH values. The porous PCL microspheres show a pH dominated release of DOX in vitro.

Scheme 1 illustrates the detailed procedure of the fabrication of PCL porous microspheres. A typical nanoprecipitation occurred when NaOH aqueous solution (0.22 M, 40 mL) was added into PCL/THF solution (1.0 mg/mL, 8 mL) at 40 ˚C. After vigorous shake, the suspension was kept in 40 ˚C water bath for 1 day. Then, large amount of DI water was added to dilute the suspension, and the porous particles were obtained after removing THF at 30 ˚C. Fig. 1 and Fig. S1 (see ESI†) show the morphological characterizations of cage-like PCL particles. The average diameter of the obtained particles is 2.6 μm (Fig. S2, ESI†). These particles contain many cavities with different sizes. High-magnified SEM and TEM images show that these cavities are multi-interconnected compartments. The size of polymer particles fabricated through nanoprecipitation method can be tuned by changing polymer concentration. Smaller PCL porous microparticles can be fabricated through decreasing the concentration of PCL solution (Fig. S3, ESI†).

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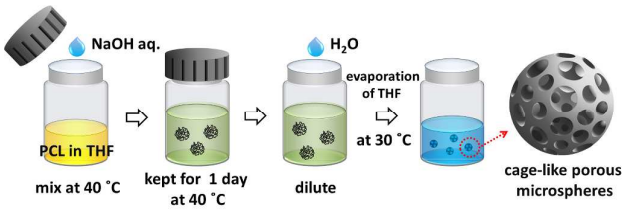


Scheme 1. Schematic illustration of the fabrication procedure of PCL porous microparticles.

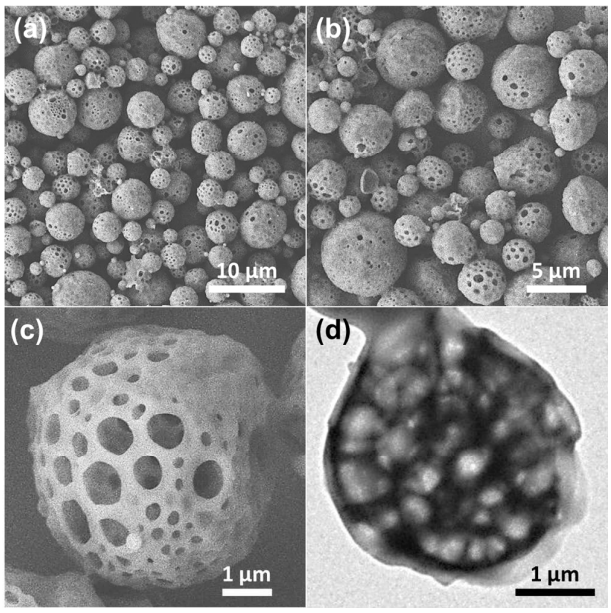


Fig. 1 Morphological characterizations of PCL porous microparticles, SEM (a-c) and TEM (d) images.

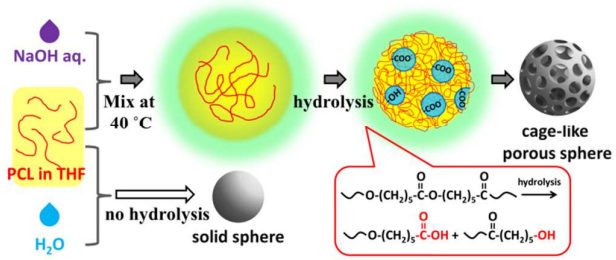
Fabrication of polymer particles through nanoprecipitation is extremely simple and attractive due to its surfactant-free feature. In general process, water (nonsolvent) is added to a water-miscible organic solvent in which polymers are dissolved. Spontaneous emulsion leads to the generation of small oil droplets, and finally polymers precipitate as nanoparticles. However, it is hard to fabricate porous structure directly by nanoprecipitation based on its mechanism.[10] We have conducted normal nanoprecipitation of PCL using water as nonsolvent. Solid PCL microspheres are generated as expected (Fig. S4, ESI†). The formation of cage-like PCL microspheres via nanoprecipitation is unusual. It attracts us to explore the underlying formation mechanism.

Polycaprolactone, a kind of polyester, can be hydrolyzed under alkali condition.[11] In our experimental condition, PCL precipitated by 0.22 M NaOH at 40 ˚C for 1 day may be partially hydrolyzed. Why the hydrolysis in nanoprecipitation influence the morphology of PCL microspheres? A series of chemical characterizations were conducted to clarify it. Table S1 (see ESI†) shows the molecular weights of porous PCL microspheres and raw PCL measured via Gel Permeation Chromatography (GPC). Compared with raw material, the molecular weight of porous PCL microspheres is near a third of raw material, and its polydispersity index (Mw/Mn) becomes broadened, showing a breakage of PCL chains after hydrolysis. ^{1}H NMR characterization shows that after hydrolysis the basic molecular structure of PCL remains, but the terminal hydroxyl group (-CH_2OH) created by hydrolysis is appearing (Fig. S5a, ESI†). The melting temperatures of cage-like porous microspheres and raw PCL material were determined by using Differential Scanning Calorimetry (DSC) (Fig. S5b, ESI†). Unlike raw PCL, cage-like microspheres show double melting peaks, which can be attributed to two different populations of crystallites. Chains in porous PCL microspheres are partially hydrolyzed, and segregate into two parts in crystallization that show two melting temperatures at 57 and 59.6 ˚C. The latter one is similar to that of raw PCL which presents melting peak at 59.5 ˚C. Zeta potential of porous PCL microspheres in different pH values is showed in Fig. S5c (see ESI†). It is observed that surface charge of microspheres changes from positive to negative at pH value around 3~5 due to the presence of abundant carboxyl groups. Under acidic environment (pH<5), carboxyl groups are protonated. When the pH increases from weak acid to physiological environment, porous microspheres are negatively charged. This specific feature of porous PCL particles provides us a good opportunity to tune surface charge of particles by changing pH value.

It is worth noting that these carboxyl end groups enable PCL chains with hydrophilic feature. In our experiments, no surfactant was added, but the product was similar to that from double emulsion. So we propose that hydrolyzed PCL may have amphiphilic property and play as surfactant to stabilize these complex droplets like that in double emulsion cases. NaOH aqueous solution is a nonsolvent for PCL. When two phases are mixed, PCL chains shrink and aggregate into droplets, forming highly swollen oil-rich phase. NaOH may be included in these droplets to induce hydrolysis of PCL. Keeping the suspension at 40 ˚C for 1 day provides enough time to partially hydrolyze PCL. The hydrolyzed product contains hydrophilic hydroxyl and carboxyl groups, and they may migrate to O/W interface to stabilize the inside water domains like a surfactant, thus preventing the coalescence of water-rich phase inside droplets (Scheme 2). The double-emulsion structures are confirmed by characterization of optical microscope (Fig S6, see ESI†). After one day, DI water was added to quench the structure and further evaporation of THF preserves porous structure. As a contrast, a batch of particles was prepared, in which evaporation of THF immediately follows the solution mixing without keeping at 40 ˚C for 1 day. Fig. S7 (see ESI†) shows that the particles were solid with small pits or with one big cavity. We measured the concentration of carboxyl group on PCL microspheres by conductometric titration method.[12a] Two kinds of PCL microspheres were tested: cage-like porous PCL microspheres and particles with small pits or with one big cavity (NaOH as nonsolvent, without being kept for 1 day under 40 ˚C). The concentrations of carboxyl group on these particles are 0.25 mmol/g and 0.16 mmol/g, respectively. These data suggests that the generation of double-emulsion-like porous structure requires a certain amount of carboxyl groups for stabilizing interface.

Moreover, we found that mixing temperature dramatically influences the morphology of PCL microspheres. When PCL/THF solution and NaOH aqueous solution are mixed at 20 ˚C, obtained microspheres are mixture of cage-like and plate-like microspheres as shown in Fig. S8b (see ESI†). The proportion of plate-like microspheres increases with decreasing mixing temperature,

almost all microspheres are plate-like when mixing temperature is down to 4 ˚C (Fig. S9, ESI†). Such plate-like morphology is a typical feature of crystallized PCL.[12b] The mixing temperature may dominate the conformation of PCL chains, which is flexible at higher temperature and rigid at lower temperature. These rigid chains are easier to crystallize when solvent property changes, resulting in plate-like PCL microspheres in nanoprecipitation.



Scheme 2. Formation mechanism of cage-like PCL microspheres.

Polycaprolactone is suitable for controlled drug delivery due to high permeability to many drugs, excellent biocompatibility and its ability to be fully excreted from the body once bioresorbed.[13] In general, drug release kinetics of PCL microspheres-based systems is determined by degradation of PCL itself. As a consequence, the drug will burst into release once polymer degrades. It is highly desirable to change this drug-release pattern to stimuli-controllable kinetics. Adsorption is a noninvasive drug loading technology that involves hydrogen bonding and electrostatic interaction.[14] Based on zeta potential values of porous PCL microsphere, we found that pH value shows great influence to the surface charge of PCL microspheres, which will change the electrostatic interaction between negatively charged PCL and positively charged molecule like DOX.

We have examined the drug storage and release of porous PCL microparticles in vitro. These porous PCL microspheres show large porosity (87 ± 5%). Fig. 2a shows fluorescent image of the porous microspheres containing DOX. The loading efficiency of porous microspheres is 36.8%, which is significantly higher than that of traditional nanoprecipitation.[10a] The high loading capacity of microspheres can be attributed to its high specific surface area and plentiful carboxyl groups at surface. The in vitro release of DOX from porous PCL microspheres was examined in phosphate-buffered solutions (PBS) with different pH values. Fig. 2b shows that the DOX release rate from porous microspheres is pH-dependent. In PBS (pH 7.4) similar to normal physiological conditions, less than 8% DOX was released from microspheres within 30 hours. However, in PBS at pH 5.0 which simulates the intracellular conditions of cancer cells, the release rate of DOX from microspheres became much faster. The cumulative release of DOX from microspheres reaches 70% within 30 hours. Under acidic condition (pH 5.0), the carboxyl groups are partially deionized, which diminishes the electrostatic interaction between protonated DOX molecules and $-COO^-$ groups, resulting in pH-controllable release of DOX. These results demonstrate that porous PCL microspheres can be a very promising drug delivery system for pH controllable drug release. As a contrast, we fabricated DOX-loaded solid PCL microspheres by conventional nanoprecipitation in which water is used as nonsolvent. The loading efficiency is 4.1%, which is similar to that reported in literature.[10a,15] The efficiency of drug incorporation into nanoparticles is generally limited by drug solubility in water. Due to the hydrophilic property, DOX•HCl tends to stay in the aqueous phase instead of being embedded into particles during nanoprecipitation. The in vitro release of DOX from solid microspheres was also examined in pH 5.0 and pH 7.4 PBS buffers. Under acidic condition, the release rate of solid microspheres is slower than that of porous. In pH 7.4 buffer, the drug release profiles of solid and porous microspheres are similar.

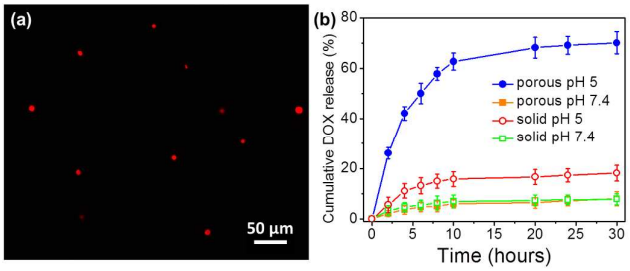


Fig. 2 (a) Fluorescent image of porous microspheres containing DOX. (b) Drug release from PCL porous and solid microspheres in different PBS (pH 5.0 and pH 7.4).

In summary, we demonstrated a new application of nanoprecipitation method in fabrication of hierarchical porous microspheres. The nanoprecipitation of PCL combines with in situ hydrolysis by using NaOH aqueous solution as nonsolvent, leading to the generation of porous microspheres in a soap-free process. Cage-like and plate-like porous PCL microspheres were obtained at different mixing temperatures. Characterizations by GPC, ^{1}H NMR, DSC, and zeta potential indicated that PCL were partially hydrolyzed. Hydrolyzed chains with hydroxyl and carboxyl end groups play as surfactant in nanoprecipitation, resulting in a double-emulsion-like result. Moreover, porous microspheres can be used for noninvasive electrostatic loading and controlled release of DOX. DOX release shows pH-dominated kinetics: less than 8% within 30 hours at pH 7.4, and up to 70% within 30 hours at pH 5.0. These porous PCL microspheres could be a very promising drug delivery system for pH controllable drug release.

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graphic abstract

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