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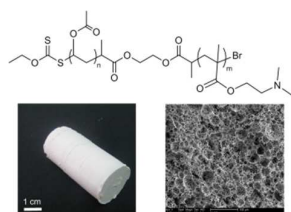
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Cationic amphiphilic surfactant PVAc-*b*-PDMAEMA has high emulsifying-capacity for CO₂/H₂O system, which can be used to prepare highly porous emulsion-templated materials.

Cite this: DOI: 10.1039/c0xx00000x

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ARTICLE TYPE

Synthesis of CO₂-philic Poly(Vinyl Acetate)-Based Cationic Amphiphilic Surfactant by RAFT/ATRP and its Application in Preparing Monolithic Materials

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5 Received (in XXX, XXX) Xth XXXXXXXXXX 20XX, Accepted Xth XXXXXXXXXX 20XX

DOI: 10.1039/b000000x

This study provides a new method to prepare CO₂-philic amphiphilic surfactants by copolymerizing less activated monomer (VAc) with activated monomer (DMAEMA). Initially, the difunctional RAFT agent *S*-(1-methyl-4-hydroxyethyl acetate) *O*-ethyl dithiocarbonate, containing the xanthate group and 2-bromopropionyl group in its structure was synthesized. By successive living radical polymerization, a series of new cationic surfactants poly(vinyl acetate)-*block*-poly(dimethylaminoethyl methacrylate) (PVAc-*b*-PDMAEMA) was obtained. PVAc-*b*-PDMAEMA cationic surfactants can emulsify CO₂/H₂O systems effectively to get high internal phase emulsion that remains stable up to 12 h. Further study indicates that the emulsifying ability of the surfactant is affected by the pH of solution and pressure etc. After the high internal phase CO₂-in-water (40% w/v, acrylamide solution) emulsion was polymerized, polyacrylamide-based highly porous emulsion-templated materials were obtained with tunable size and interconnected pores. These porous materials were then used as scaffold to guide cellular growth.

Introduction

In general, porous organic polymers can be prepared by polymerizing a high internal phase emulsion (HIPE) (>74%, v/v internal droplet phase). Subsequent removal of the internal phase gives rise to a porous replica of the emulsion.^{1, 21} This method is usually used as a versatile method to prepare highly porous organic polymers,^{3, 4} inorganic materials⁵⁻⁷ and inorganic-organic composites.^{8, 9} These materials can be widely used in the fields of catalysis, biological scaffolds¹⁰ and drug delivery¹¹ etc. The porous materials preparing from polyHIPE combine a fully interconnected, highly porous structure, a low bulk density, a high permeability, and a high surface area with decent mechanical integrity.¹² In principle, the polymerization of concentrated oil-in-water (O/W) emulsions also provides a direct synthetic route to a variety of porous hydrophilic polymers.¹³ However, this method has a significant disadvantage that the residual organic solvent may be difficult to remove from the materials at the end of the reaction. The residual organic solvent may be disadvantageous for the tissue engineering or other applications due to their toxic nature. Liquid or supercritical carbon dioxide (sc-CO₂) is a non-toxic, non-flammable, non-expensive, natural solvent and can be used as an alternative to conventional organic solvents for the preparation of

40 high internal phase CO₂-in-water (C/W) emulsions. With the exception of fluorinated silicone-based polymers, CO₂ lacks the solvent power to solvate macromolecules easily.¹⁴⁻¹⁷ Although these fluorinated or silicone-based materials have been studied as steric stabilisers in dispersion polymerization¹⁸⁻²⁰ and as surfactants in preparing C/W or water-in-CO₂ (W/C) emulsions or micro-emulsions,²¹⁻²⁷ the associated cost and poor environmental degradability may prohibit their industrial-scale applications. In recent years, design and synthesis of CO₂-philic hydrocarbon-based polymers have received significant attention. Poly(ether-carbonate) (PEC),²⁸ poly(ether ester) (PEE),²⁹ especially PVAc-based homopolymers or copolymers, have been reported to possess strong CO₂-philic character and have low-cost in addition to being environmental friendly.³⁰⁻³³ Based on these materials, some non-ionic surfactants have also been developed.^{34, 35} Johnston investigated a series of commercially available alkylene oxide-based surfactants, which can form very stable and concentrated C/W emulsions at 22-65 °C with CO₂ volume fractions up to 70%.³⁶ Rocha synthesized several triblock copolymers from DL-lactide and poly(ethylene glycol), which showed high activity at the C/W interface even at low (0.01 wt%) overall surfactant concentration.³⁷ In our previous study, di-/tri-block polymers from oligo(vinyl acetate) (OVAc) and poly(ethylene glycol) (PEG) were

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†Electronic Supplementary Information (ESI) available: ¹³C NMR analysis of analysis of *S*-[1-Methyl-4-(6-Bromopropionate) Ethyl Acetate] *O*-Ethyl Dithiocarbonate; GPC traces of PVAc-Br.

synthesized, which showed remarkable emulsification properties, and were able to emulsify up to 97% (v/v) C/W.³⁸⁻⁴⁰ In comparison, ionic hydrocarbon-based surfactants also show excellent emulsifying-capacity in CO₂/H₂O systems, but their use to prepare the stable emulsions or micelles is very limited.⁴¹ In 2005, Fan et al first synthesized the sodium bis(vinyl acetate)sulfosuccinate,⁴² and Eastoe used it as the surfactant to prepare the W/C microemulsions.⁴³ Shortly afterwards, Tan also synthesized a new PVAc-based twin-tail ionic surfactant to produce the W/C emulsions.⁴⁴ After that, a few more reports have also been published highlighting the preparation of PVAc-based ionic surfactants for the same purpose. Now we have designed and synthesized another PVAc-based ionic surfactant i.e., poly(dimethylaminoethyl methacrylate) (PDMAEMA) which is a multi-functional polymer with widespread applications in areas requiring biocompatibility, such as hydrogel and drug delivery etc.⁴⁵⁻⁴⁷ PDMAEMA contains pendant tertiary amines which are easily protonated below its pKa (7.5) and indicates a strong hydrophilic character.

An efficient surfactant should possess an appropriate ratio of hydrophilic part to CO₂-philic part.^{36, 37} Therefore a control over the molecular weight of each block is important. VAc is a less activated monomer, and many reports have verified that xanthates can better control its polymerization.⁴⁸⁻⁵⁰ However, due to the higher reactivity of VAc and (methyl)acrylate, xanthates have poor efficiency with regard to their controlled polymerisation, which limits the synthesis of particular amphiphilic block polymers or stabilizers. Li and Matyjaszewski developed a new method to prepare the block polymers of VAc and (methyl)acrylate monomers such as PVAc-*b*-PSt, PVAc-*b*-PMA, or PVAc-*b*-PMMA by successive reversible addition-fragmentation chain transfer radical polymerization (RAFT) and atom transfer radical polymerization (ATRP) with a bromoxanthate iniferter.^{51, 52} This method provides a good strategy to synthesize some particular amphiphilic surfactants.

Here, we report the use of the combination of RAFT and ATRP methods to synthesize the PVAc-*b*-PDMAEMA amphiphilic copolymers by using a bromoxanthate iniferter as the chain transfer agent (CTA) and initiator (ATRP). The results indicate that PVAc-*b*-PDMAEMA copolymers are effective surfactants to prepare stable CO₂-in-water emulsions, and their emulsifying capacity can be adjusted by controlling the molecular chain length or pH of the solution. Polymerization of emulsion-templating formed by AM solution/CO₂ system, PAM monolithic materials were obtained. At last, the monolithic materials was used as scaffolds to guide cell growth, and the results indicate that the cells can grow well in the inner walls of the materials within 48h demonstrating the potential applications of such materials in tissue engineering.

Experimental Sections

Materials

Vinyl acetate (VAc, Sinopharm, 97%) and 2-(dimethylamino)ethyl methacrylate (DMAEMA, Aladdin, 99%) were freed from inhibitor, and further purified by reduced pressure distillation over calcium hydride. 2, 2'-azobis(isobutyronitrile) (AIBN, Sinopharm, 80%) was recrystallized twice from methanol. Ammonium persulfate (APS, Sinopharm, 99%) was recrystallized twice from

water. CuCl (Aladdin, 98%) was purified by stirring in acetic acid, washing with ethanol and ether. 2-bromopropionyl bromide (98%), triethylamine (99%), ethylene glycol (99%), acetone (99.5%), potassium hydroxide (KOH, 82%), anhydrous magnesium sulfate (99%), tetrahydrofuran (THF, 99%), dichloromethane (99%), carbon disulfide (99%), ethanol (99%), anisol (99%) acrylamide (AM, 98%) and *N,N*-methylene bis(acrylamide) (MBAN, 98%) were all purchased from Sinopharm, and used as received. 1,1,4,7,10,10-hexamethyltriethylenetetramine (HMTETA),⁵³ potassium *O*-ethyl dithiocarbonate⁵⁴ and *S*-(1-methyl-4-hydroxyethyl acetate) *O*-ethyl dithiocarbonate (X-Br)⁵¹ were synthesized according to the literature (Fig. 1a, Fig. S1). High purity carbon dioxide (99.99%) was purchased from Ming Hui Gas. Glutaric dialdehyde (GA, Medical Grade), Na-cacodylate, sucrose (Medical Grade) and hexamethyl disilylamine (HMDS, Medical Grade) were purchased from Sigma-Aldrich.

General Procedure for RAFT Polymerization of Vinyl Acetate

A typical polymerization protocol that was adopted is summarized: VAc (10.8 g, 0.126 mol), X-Br 1.20 g, 3.22×10^{-3} mol), AIBN (0.263 g, 1.61×10^{-3} mol) and THF (5.50 g) were placed in a 50 ml three-neck flask by passing nitrogen through the flask for half an hour in an ice-bath. The flask was immersed in a water-bath preheated at 65 °C with stirring for 5 h. The mixture was diluted by THF, and re-precipitated twice in cold n-hexane. The product (PVAc-Br) was dried under vacuum at 35 °C. M_n -GPC: 1250, PDI: 1.43, M_n -NMR: 1660.

Typical Procedure for the Synthesis of PVAc-*b*-PDMAEMA by ATRP Method

A typical polymerization protocol that was adopted is summarized: the mixture of PVAc-Br (1.00 g, 3.39×10^{-4} mol; M_n -GPC: 1250, PDI: 1.31), CuCl (33.5 mg, 3.39×10^{-4} mol), HMTETA (23.5 mg, 1.0×10^{-4} mol), DMAEMA (3.00 g) and anisol (6.0 mL) were degassed by three freeze-pump-thaw nitrogen cycles, sealed off under vacuum, and immersed into a water-bath preheated at 50 °C with stirring for 3 h. The final mixture was diluted with THF. After removing the remaining copper compounds by passing the final block copolymers through a column containing silica gel, the solution was re-precipitated into cold n-hexane for three times. The product was dried under vacuum at 35 °C. M_n -GPC: 2220, PDI: 1.46, M_n -NMR: 4270.

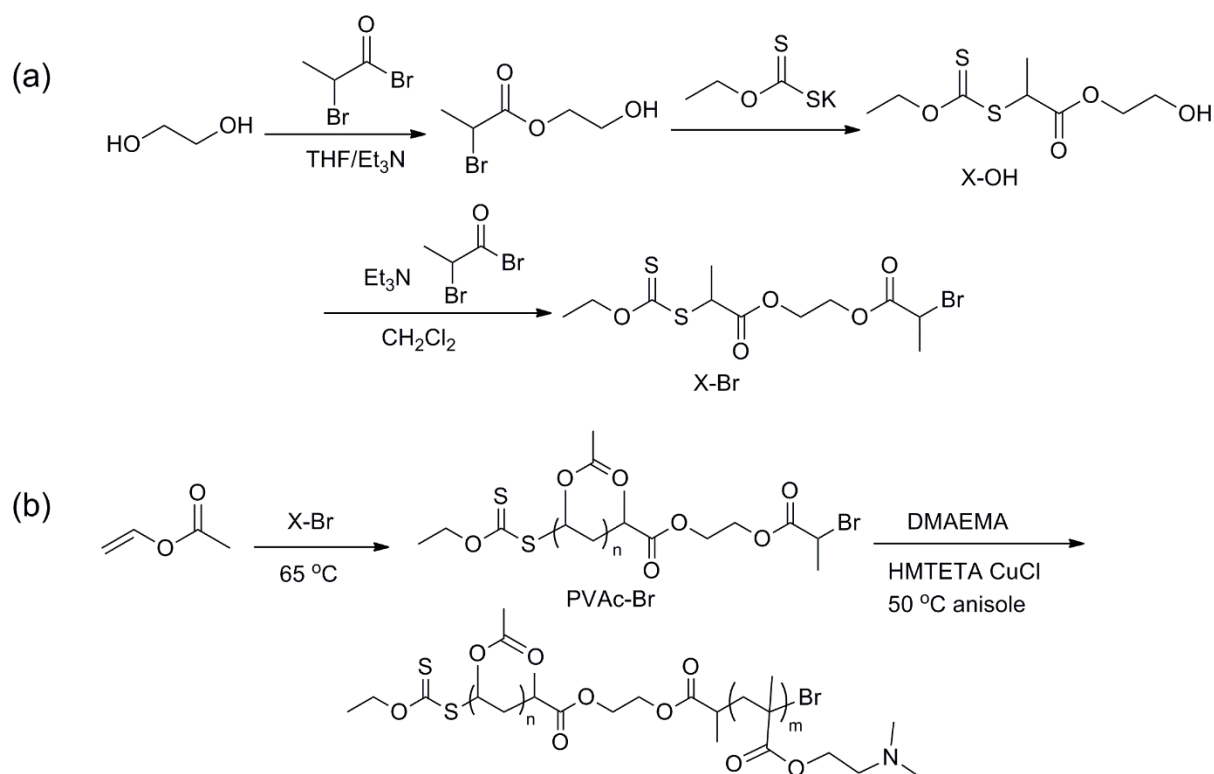
Polymerization of C/W Emulsions

In a typical polymerization, an aqueous solution of AM/MBAN(4/1 w/w, 40% w/v), tetramethyl ethylenediamine (TMEDA, 0.10 mL) and surfactant were added into a 10 cm³ stainless steel view cell reactor. The reactor was purged with a flow of CO₂ for 3 min to eliminate any oxygen, and then pressurized with CO₂ (15 °C, 12 MPa). The mixture was stirred with a stirring-bar (600 rpm) until forming a stable milky C/W emulsion. Ammonium persulfate (0.040 g in 0.1 mL water, 2% w/v based on monomer) was added into the cell, and stirred for 5 min. After 12 h at room temperature, the CO₂ was slowly vented (60 min). The product was recovered as a continuous white monolithic sample that conformed to the internal dimensions of the reactor. Subsequently, the material was immersed into water to remove excessive initiator and monomers, and then freeze-

Cite this: DOI: 10.1039/c0xx00000x

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Scheme 1 Synthesis of difunctional chain transfer agent X-Br (a) and PVAc-*b*-PDMAEMA block copolymer (b).

dried using a Scientz-12N freeze-dryer for more than 24 h.

Cell Culture

5 The human embryonic lung diploid fibroblast cells were cultured on PAM-based monolithic materials with 3D structure. Firstly, the materials (slices with < 0.5 cm in thickness) were placed into a 48-well plate, and sterilized with ethanol twice. The ethanol contained inside the slices was replaced by culture media (DMEM, 10% FBS, pen-strep), and the slices were equilibrated in culture media under

10 37 °C for 12 h.

Fibroblast cell suspension (50 μL, 2.5 × 10⁵ cells) was then added drop-wise onto the surface of slices, and then the slices were placed in the incubator for 30 min to allow equilibrium swelling that initiates the cell attachment to the walls of slices. After that, more basal medium was added to make the whole slices dispersed, and the cells loaded on the slices were cultured in CO₂ atmosphere at 37 °C for 48 h. Finally, the slices were dispersed in fixative solution (2% v/v GA which contains 0.1 M Na-cacodylate buffer and 0.1 M sucrose) for 2 h, and then the slices were dehydrated using graded ethanol (70%, 80%, 90%, 100%), and dried by using HMDS.

Characterization

Nuclear magnetic resonance (NMR) spectra were recorded in CDCl₃ on a Bruker AV400 MHz spectrometer at room temperature

using tetramethylsilane (TMS) as an internal reference. The morphology of the monolithic materials was investigated with an FEI Sirion 200 field emission scanning electron microscope (FE-SEM). For SEM analysis, samples were mounted on aluminium stubs using adhesive graphite tape and sputter-coated with gold before analysis. Gel permeation chromatography (GPC) was performed with an Agilent 1100 instrument using refractive index detector (RID) and a chromatography column (PLgel 5 μm), THF was used as an eluent at a flow rate of 1.0 mL/min at 30 °C. The calculated molecular weights were based on a calibration curve for polystyrene (PS) standards of narrow polydispersity (Polymer Laboratories). Pore size analysis was carried out by ImageJ 1.44p software, and the size was calculated by the following equation:

$$d = 2\sqrt{S/\pi} \quad (S: \text{the average area of the porosity}; d: \text{the average diameter}).$$

Results and Discussion

Synthesis of the macromolecular initiator OVAc-Br

Firstly, a bromoxanthate iniferter including *O*-ethyl xanthate moiety and bromo- moiety was synthesized, which can control the polymerization of VAc by RAFT, or initiate the polymerization of some monomers ((meth)acrylates or styrene monomers) by ATRP. It seems that the PVAc-*b*-PDMAEMA block copolymer can be

obtained by two different strategies: ATRP of DMAEMA was conducted either before or after the RAFT polymerization of VAc (Scheme 1). Li and Matyjaszewski have reported that first RAFT of VAc, and then ATRP of conjugated monomers can get the perfect block copolymer by using PVAc-Br as macro-initiator.^{51, 52} The molecular weight of each block was determined after appropriate time intervals. In order to investigate the effect of the molecular weight of CO₂-philic part on the stability of CO₂-in-water emulsion, three

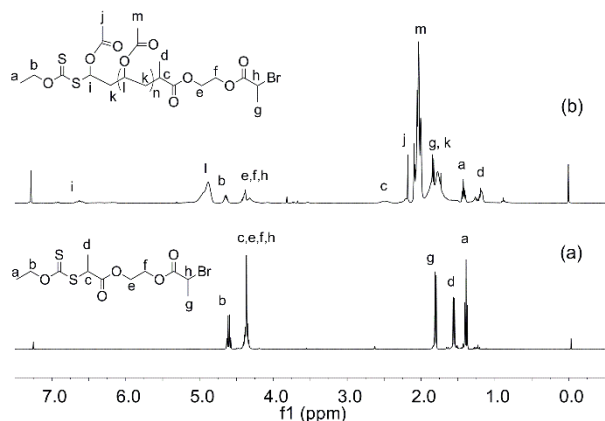


Fig. 1 ¹H NMR analysis (CDCl₃) of S-[1-Methyl-4-(6-Bromopropionate) Ethyl Acetate] O-Ethyl Dithiocarbonate (a), and (PVAc)₁₆-Br (b).

Table 1 GPC and ¹H NMR analysis data of three PVAc-Br using X-Br as CTA.

Time (h)	Conv. (%)	$M_{n,th}$	$M_{n,GPC}^a$	$M_{n,NMR}^b$	PDI	DP	First Block
3 h	12	1100	940	1150	1.46	9	(PVAc) ₉ -Br
5 h	24	1800	1250	1780	1.43	16	(PVAc) ₁₆ -Br
10 h	33	2300	2390	2380	1.52	22	(PVAc) ₂₂ -Br

^a Gel permeation chromatography (GPC) in tetrahydrofuran with PS standards; ^b calculated by the following equation: $M_{n,NMR} = 2 \times \left(\frac{I_{4.78-5.04} + I_{6.54-6.69}}{I_{4.56-4.69}} \right) \times M_{VAc} + M_{X-Br}$

OVAc with different molecular weight were synthesized in bulk at 65 °C. The results (Table 1) show that the M_n of OVAc ranges from 940 to 2390 with narrow PDI (<1.5), whose chromatograms (Fig. S2) show single peak and symmetrical traces especially at low VAc conversion.

According to the RAFT mechanism, the X-Br moieties should exist in the final products, that is evident from ¹H NMR spectrum (Fig. 1). The characteristic chemical shifts of the X-Br corresponding to the protons of b, c, g, d are observed at 4.61, 4.37, 1.80 and 1.56 ppm respectively (Fig. 1(a)). In the corresponding spectrum of PVAc-Br (Fig. 1(b)), the hydrogens b and g retain the same chemical shifts, but the hydrogens c, d are shifted to 2.21 and 1.19 ppm respectively. The methine protons belonging to the repetitive PVAc units are observed at 4.87 ppm (l) and 6.64 ppm (i, which are connected with O-ethyl dithiocarbonate moieties). These results confirm that the CTA moieties are attached to PVAc. The number-average molecular weight ($M_{n,NMR}$) can be calculated according to the integral of the methine protons of OVAc moiety and the methylene protons of the RAFT moiety. All the $M_{n,NMR}$ data are listed in Table 1, which is in accordance with $M_{n,th}$.

Synthesis of PVAc-*b*-PDMAEMA Block Copolymers

In the structure of PVAc-Br, the end group moiety is a secondary acrylate end containing a bromine atom. When used as macromolecular initiator by ATRP, it will produce a secondary radical to initiate the polymerization of DMAEMA, and thus the PVAc-*b*-PDMAEMA amphiphilic block polymer is synthesized. But a question arises that if CuBr is used as a catalyst, the chain growth will be faster than the initiating rate, that leads to some unreacted macroiniferter. Matyjaszewski improved the initiator

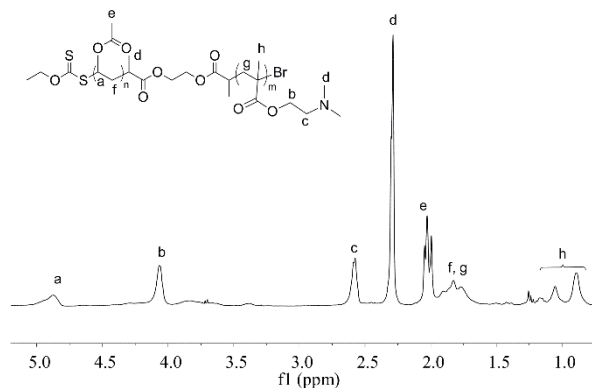


Fig. 2 ¹H NMR analysis (CDCl₃) of (PVAc)₁₆-*b*-(PDMAEMA)₁₇

efficiency relative to the rate of propagation through halogen exchange by using CuCl instead of CuBr as the catalyst.⁵⁵ We also adopted the same strategy, and HMTETA was used as the ligand. Fig. 2 shows a typical example of ¹H NMR spectra of the block copolymer. The typical chemical shifts of PVAc (a, e) and PDMAEMA (b, c) are observed clearly. According to the NMR spectra, the DP of PDMAEMA can be calculated by comparing the integration ratio of -CH₂CH₂N(CH₃)₂ and methine of PVAc. This block copolymer is noted as (PVAc)_x-*b*-(PDMAEMA)_y (x, y represent the degree of polymerization), and all data are listed in Table 2. Using three PVAc-Br macromolecular initiators with different M_n , a series of amphiphilic block copolymers was synthesized.

With an increase in the conversion of DMAEMA, the theoretical molecular weight ($M_{n,th}$) is almost in accordance with $M_{n,NMR}$ (Table 2). At the first initiating stage, the initiator efficiency is low. However, as the bromine is exchanged with a less labile chlorine for the dormant chain which decreases the apparent rate constant of propagation, the initiator efficiency is significantly improved. It is also reflected from the PDI (GPC) (ranging from 1.83 to 1.54).

Emulsion Stability

Tertiary amine can be protonated especially at low pH and shows a strong hydrophilic character. Johnston investigated several tertiary esters as surfactants for CO₂-based dispersions to stabilize C/W macroemulsions.⁵⁶ The combination of a weakly hydrophilic tertiary amine which is protonated by carbonic acid, and the branched ester tails provide appropriate hydrophilic-CO₂-philic balance for emulsion stabilization. The emulsion remained stable even at a CO₂ density as low as 0.74 g/mL (70 bar). But the emulsions stability is maintained only for a few hours under high pressure (34.5 Mpa). Furthermore their stability decreases greatly by an increase in pH owing to the short hydrophilic moiety. For the surfactant we synthesized, when the DP of PVAc is less than 9, there were no stable emulsions formed even at pressure up to 25

MPa (Table 3, entry 1 and entry 2). When the DP of PVAc increases to 16, the highly concentrated milky-white C/W emulsions were formed. These findings indicate that an appropriate hydrophilic/CO₂-philic ratio is a key factor for the

stability of emulsion. When using (PVAc)₁₆-*b*-(PDMAEMA)₁₇ as the surfactant, the emulsion can remain stable for 12 h under static conditions (Table 3, entry 4). Surprisingly the HIPE is still

Table 2 Molecular weight characteristics of the block copolymers.

First Block	Conv. ^a (%)	$M_{n,th}^b$	$M_{n,GPC}$	$M_{n,NMR}^c$	PDI	DP	Block copolymers
(PVAc) ₉ -Br	23	3120	1510	3350	1.66	14	(PVAc) ₉ - <i>b</i> -(PDMAEMA) ₁₄
	46	4130	2700	4290	1.43	20	(PVAc) ₉ - <i>b</i> -(PDMAEMA) ₂₀
(PVAc) ₁₆ -Br	32	3570	1750	3700	1.83	13	(PVAc) ₁₆ - <i>b</i> -(PDMAEMA) ₁₃
	53	4130	2220	4270	1.46	17	(PVAc) ₁₆ - <i>b</i> -(PDMAEMA) ₁₇
	66	4690	2300	4960	1.58	21	(PVAc) ₁₆ - <i>b</i> -(PDMAEMA) ₂₁
	83	5460	2670	5740	1.54	26	(PVAc) ₁₆ - <i>b</i> -(PDMAEMA) ₂₆
(PVAc) ₂₂ -Br	18	4580	2970	4700	1.66	19	(PVAc) ₂₂ - <i>b</i> -(PDMAEMA) ₁₉

^a the conversion was calculated by weight method; ^b $M_{n,th}$: $M_{n,th} = \left(\frac{x[M]_0}{[PVAc-Br]_0} \right) \times M_{DMAEMA} + M_{PVAc-Br}$; ^c $M_{n,NMR}$: $M_{n,NMR} = n \times \left(\frac{1_{2,16-2,29}}{1_{4,69-5,06}} \right) \times M_{DMAEMA} + M_{PVAc-Br}$ (n: the DP of PVAc - Br).

Table 3 Stability of C/W emulsion prepared using PVAc-*b*-PDMAEMA as surfactants.

Entry	Surfactants	% (w/v)	C/W (v/v)	Emulsion Stability ^b
1	(PVAc) ₉ - <i>b</i> -(PDMAEMA) ₁₄	1.0	8:2	Partial emulsion, 12-25 MPa
2	(PVAc) ₉ - <i>b</i> -(PDMAEMA) ₂₀	1.0	8:2	Partial emulsion, 12-25 MPa
3	(PVAc) ₁₆ - <i>b</i> -(PDMAEMA) ₁₃	1.0	8:2	Stable emulsion, 12 MPa 6.5 h
4	(PVAc) ₁₆ - <i>b</i> -(PDMAEMA) ₁₇	1.0	8:2	Stable emulsion, 12 MPa 12 h
5	(PVAc) ₁₆ - <i>b</i> -(PDMAEMA) ₁₇	0.30	8:2	Stable emulsion, 6.6 MPa 2 h
6	(PVAc) ₁₆ - <i>b</i> -(PDMAEMA) ₁₇	0.30	8:2	Stable emulsion, 12 MPa 4.0 h
7	(PVAc) ₁₆ - <i>b</i> -(PDMAEMA) ₁₇	0.16	8:2	Stable emulsion, 12 MPa 1.5 h
8	(PVAc) ₁₆ - <i>b</i> -(PDMAEMA) ₁₇	1.0	9:1	Stable emulsion, 20 MPa, 16 h
9	(PVAc) ₁₆ - <i>b</i> -(PDMAEMA) ₂₁	1.0	8:2	Stable emulsion, 12 MPa 7.0 h
10	(PVAc) ₁₆ - <i>b</i> -(PDMAEMA) ₂₁	1.0	9:1	Partial emulsion, 12-25 MPa
11 ^a	(PVAc) ₁₆ - <i>b</i> -(PDMAEMA) ₂₁	1.0	9:1	Stable emulsion, 20 MPa 6.0 h
12	(PVAc) ₁₆ - <i>b</i> -(PDMAEMA) ₂₆	1.0	8:2	Stable emulsion, 25 MPa 6.5 h
13	(PVAc) ₂₂ - <i>b</i> -(PDMAEMA) ₁₉	1.0	8:2	Stable emulsion, 12 MPa 3.0 h

^a two drops HCl solution (1 mol/L); ^b An aqueous solution of AM + MBAM (40% w/v in H₂O, AM/MBAM = 8:2 w/w) without any additives; conditions: 15 °C, 12~25 MPa.

formed even if the concentration of surfactant decreases to 0.16%(w/v), and can remain stable for at least 1.5 h (Table 3, entry 7). These results indicate that PVAc-*b*-PDMAEMA is an efficient surfactant to prepare highly stable and concentrated C/W emulsions without any additive.

In our previous study, we found non-ionic amphiphilic surfactant OVAc/PEG-based diblock or triblock polymer have high emulsifying capacity, which can form the highly concentrated C/W emulsions from 80% to 90%, even 97% (v/v, internal phase volume) under 12 MPa, and the emulsion can remain stable for more than 48 h. In this study, when the internal phase volume increases to 90%, however, even using the (PVAc)₁₆-*b*-

(PDMAEMA)₁₇ which has the best emulsifying capacity as the surfactant, the stable emulsion can't be formed until the pressure approaches to 20 MPa. It is well known that the pH of the solution can be adjusted by adding an acid, such as hydrochloric acid (HCl). Even though a few drops of HCl were added into the solution, the emulsifying-capacity of PVAc-*b*-PDMAEMA was also improved significantly. For example, (PVAc)₁₆-*b*-(PDMAEMA)₂₁ can't emulsify 90% (v/v, CO₂) high internal phase emulsion completely (entry 11), however, when 2 drops of HCl (1 M) were added, the highly concentrated C/W emulsions were obtained (Table 3, entry 12). So the hydrophilicity of PDMAEMA and the hydrophilic/CO₂-philic balance were improved.

In summary, above analysis suggests that OVAc-*b*-PDMAEMA is a very effective surfactant, which can be used to prepare stable and highly concentrated C/W emulsions, and its emulsifying capacity can be improved easily by pH adjustment of solution. Thus OVAc-*b*-PDMAEMA is more suitable to emulsify acidic-solution/CO₂ system.

Synthesis of PAM-based monolithic materials

According to the literature, the ionic amphiphilic surfactant PFPE has been used to produce porous materials. However, due to its low molecular weight, poly(vinyl alcohol) (PVA) has to be used as an additive to overcome the destabilization of the emulsions, or otherwise the open-cell porous-materials can't be produced.²⁴ In addition, the high cost and low biodegradability prohibit its applications. Other ionic amphiphilic surfactant such as sodium dodecylbenzenesulfonate (SDBS), sodium dodecyl sulfonate (SDS), cetyltrimethylammonium bromide (CTAB) etc. have also been used to produce the porous materials, however due to their low emulsifying capacity, a higher concentration of surfactants (as high as 10%), which is required that increases the cost of applications.

In our previous study, we synthesized a series of OVAc-PEG-based non-ionic surfactants, and produced porous materials with controlled morphology.³⁸⁻⁴⁰ In this study, we reported another versatile method to synthesize a new PVAc/PDMAEMA-based

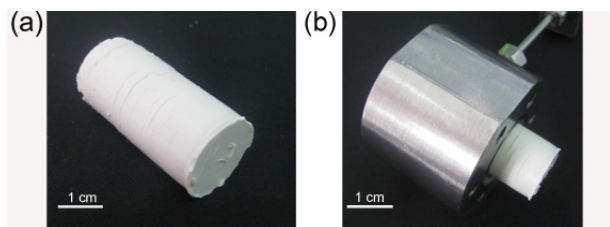


Fig. 3 Photographs of a low-density C/W emulsion templated PAM-based materials.

Table 4 Stability of C/W emulsion prepared using PVAc-*b*-PDMAEMA as surfactants.

Entry ^a	Surfactants	w/v (%)	C/W (v/v)	Diameter (μm) ^b
PAM-1	(PVAc) ₁₆ - <i>b</i> -(PDMAEMA) ₁₃	1.0	8:2	12.98 ± 0.55
PAM-2	(PVAc) ₁₆ - <i>b</i> -(PDMAEMA) ₁₃	0.60	8:2	19.08 ± 4.32
PAM-3	(PVAc) ₁₆ - <i>b</i> -(PDMAEMA) ₁₇	1.5	8:2	9.76 ± 1.63
PAM-4	(PVAc) ₁₆ - <i>b</i> -(PDMAEMA) ₁₇	1.0	8:2	8.12 ± 0.5
PAM-5	(PVAc) ₁₆ - <i>b</i> -(PDMAEMA) ₁₇	0.60	8:2	13.57 ± 1.56
PAM-6	(PVAc) ₁₆ - <i>b</i> -(PDMAEMA) ₁₇	0.30	8:2	18.48 ± 0.11
PAM-7	(PVAc) ₁₆ - <i>b</i> -(PDMAEMA) ₁₇	0.15	8:2	22.56 ± 2.88
PAM-8	(PVAc) ₁₆ - <i>b</i> -(PDMAEMA) ₂₁	1.0	8:2	12.84 ± 1.94

^a Polymerization conditions: AM + MBAM solution (40% w/v, AM/MBAM = 4/1), (NH₄)₂S₂O₈ (3%, w/v based on monomer) as initiator, and 0.10 mL TMEDA as co-initiator at 15 °C, 18 MPa; ^b analysis by ImageJ soft according to SEM analysis.

10 ionic surfactant to stabilize C/W HIEPs. According to the above discussion, PVAc-*b*-PDMAEMA surfactants can provide highly stable and concentrated C/W emulsions to form polyHIEPs. In this HIEPs system, AM + MBAM (40%, v/w) solution was used as a continuous phase, liquid CO₂ as the dispersed phase, and tetramethylene-diamine (TMEDA) as a catalytic redox co-initiator. Temperature, pressure, concentration of AM solution, and mixing speeds were kept constant. When ammonium persulfate solution was injected into the high pressure reactor, the polymerization was initiated, and the continuous phase was solidified. After removing the dispersed phase (CO₂), uniform monolithic materials conforming to the cylindrical interior of the cell were recovered, suggesting that most of the scCO₂ volume added was emulsified, and no significant shrinkage was observed after CO₂ venting (Fig. 3).

25 According to the pore forming mechanism, the pore-size is equal to the diameter of CO₂ droplets, so the morphology of the porous materials is similar to that of the original emulsions. From SEM analysis (Fig. 4), the pores are heterogeneous, that indicate the emulsion droplets are also heterogeneous, and is consistent with most of literature reports.^{38, 57} Table 4 lists all the porosity data of various monoliths. It's obvious that the average diameter of pore increases with a decrease in the concentration of surfactant. For example, the average pore diameter increases from 12.98 μm (PAM-1) to 19.08 μm (PAM-2) when the concentration of (PVAc)₁₆-*b*-(PDMAEMA)₁₃ decreases from 1.0% to 0.60%.

35 For (PVAc)₁₆-*b*-(PDMAEMA)₁₇, the average pore diameter decreases slightly at first, and then increases with the decrease of the concentration of the surfactant (Fig. 5a). When the concentration of surfactant is 1.0%, the average pore diameter is the smallest (PAM-4). The size of the dispersed phase liquid droplet tends to decrease with an increase in the concentration of surfactant, while the change in the pore size of porous materials

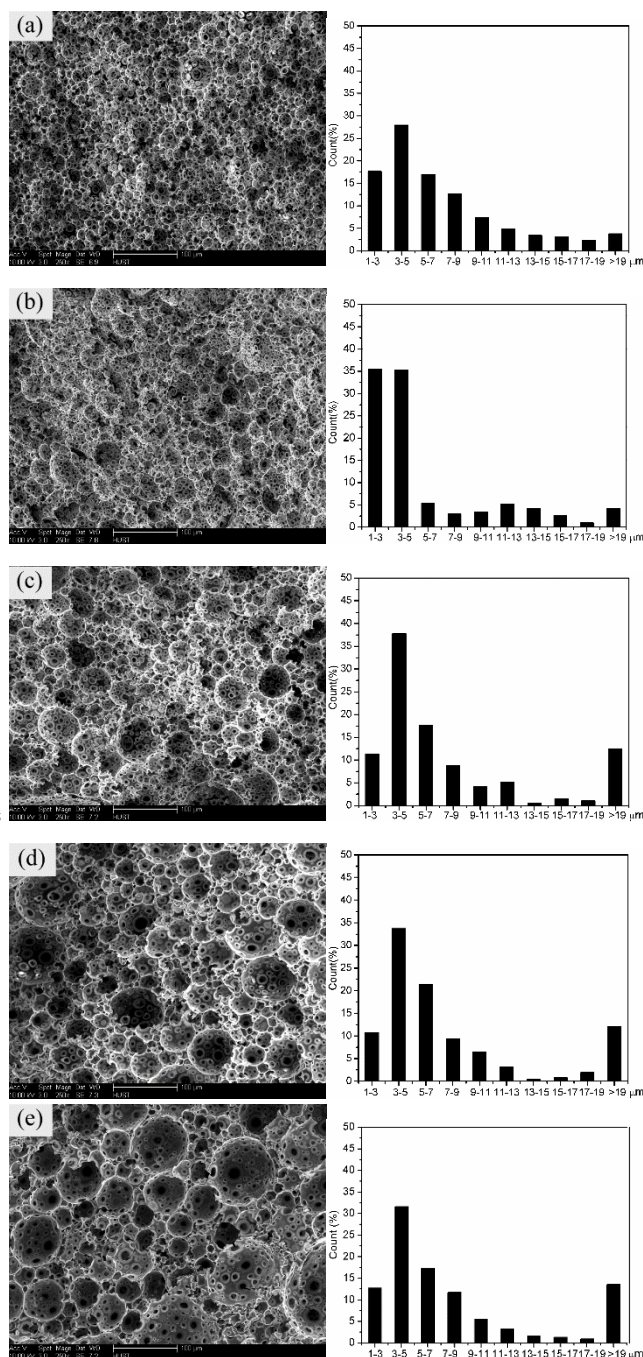


Fig. 4 SEM analysis of PAM-based macroporous materials and their pore size distributions prepared by C/W emulsion templating polymerization with various concentration of (PVAc)₁₆-*b*-(PDMAEMA)₁₇ surfactant (w/v): (a) 1.5%; (b) 1.0%; (c) 0.60%; (d) 0.30%; (e) 0.15%.

keeps the same trend under the similar conditions. This is further evident from the pore size distributions (Fig. 4). When the concentration of (PVAc)₁₆-*b*-(PDMAEMA)₁₇ is 1.0%, the pore size of the porous materials is mostly <10 μm (taking up 81% volume or so), and the percentage of macropores (>19 μm) is very low (<4%). This reflects that the size of the dispersed phase (CO₂ liquid droplets) is small. With a decrease in the concentration of the surfactant, the stability of emulsions also tends to decrease, and the CO₂ liquid droplets are prone to coalescence, leading to the formation of larger pores. As a

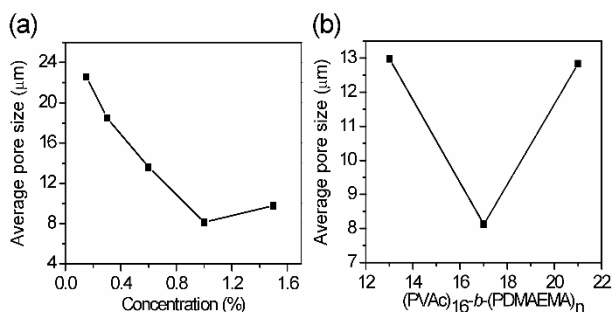


Fig. 5 (a) Effect of the concentration of (PVAc)_{16-b}-(PDMAEMA)₁₇ on the average pore size distribution; (b) Effect of DP of PDMAEMA on the average pore size distribution when keeping the DP of PVAc constant.

5 consequence of the decrease in the concentration of surfactants (1.5% to 0.15%), the macropore (>19 μm) percentage increases gradually from <4% to >13%, and these macropores are always surrounded by many smaller ones.

Keeping the DP of PVAc constant while increasing the chain length
10 of PDMAEMA, it changes the hydrophilic/CO₂-philic balance ratio. From Fig. 5b, it is still evident that using (PVAc)_{16-b}-(PDMAEMA)₁₇ as the surfactant produces monolithic materials with the smallest pore. Above all, (PVAc)_{16-b}-(PDMAEMA)₁₇ has the best hydrophilic/CO₂-philic balance ratio, which gives the best
15 emulsifying capacity.

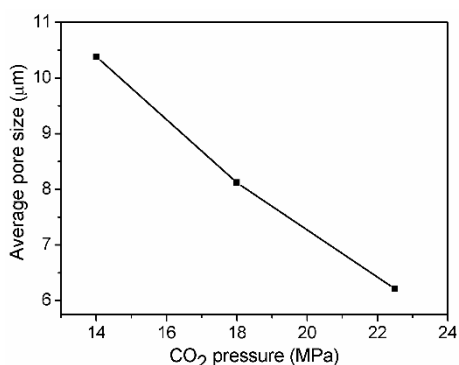


Fig. 6. The dependence of the average pore-size with CO₂ pressure while keeping the concentration of (PVAc)_{16-b}-(PDMAEMA)₁₇ constant (1%, w/v).

20 With an increase in the pressure, the PVAc shows better CO₂-philic character, thus improving the emulsifying-capacity of PVAc-*b*-PDMAEMA. The dependence of the pore size on the CO₂ pressure used is shown in Fig. 6. It is evident that the average pore size decreases by increasing CO₂ pressure. When the pressure of
25 polymerization is 15 MPa, the percentage of pore size (<10 μm) takes up only 62.7%, and the macropores (>19 μm) are over 14%. In comparison, when the pressure increases to 22.4 MPa, the percentage of pore size (<10 μm) increases to 84%, and the macropores (>19 μm) are less than 5%. In addition, the maximum
30 percentage value in distribution curves shifts to the pores with smaller size (Fig. 7, pore size distributions). All these facts show that the size of the droplet of emulsion in the C/W system tends to decrease, which reflects that the emulsifying-capacity of surfactant is improved, and emulsions become more and more stable with an
35 increase of the CO₂ pressure.

Cell culture

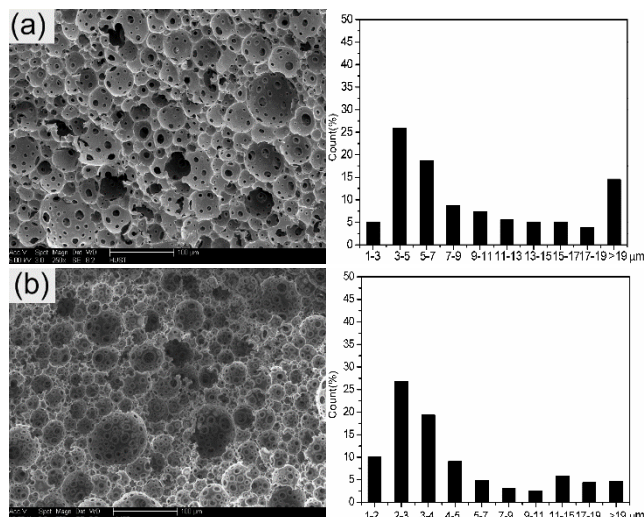


Fig. 7 Scanning electron micrographs of PAM-based macroporous materials and their pore size distributions prepared by C/W emulsion templating polymerization with CO₂ pressure (MPa) of (PVAc)_{16-b}-(PDMAEMA)₁₇ surfactant (1%, w/v): (a) 15 MPa; (b) 22.4 MPa.

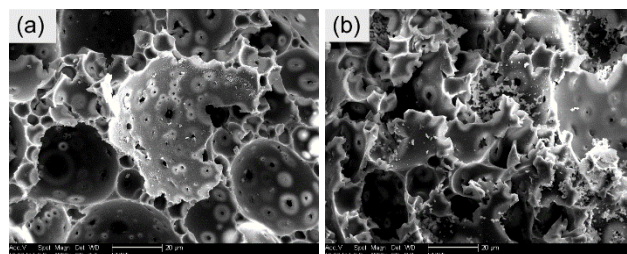


Fig. 8 SEM analysis of Cell Culture sample using PAM-8 as matrix: (a) surface; (b) inside of wall.

Tissue engineering can potentially create functional tissue constructs for patients. The development of porous materials with 3D structure is necessary to guide cellular growth as scaffold.⁵⁸⁻⁶⁰ It is an alternative method to synthesize the porous materials by
50 using liquid CO₂ as a template, which completely eliminates organic solvent. In addition, the materials obtained generally have an open-cell structure, and the corresponding pore size can be easily regulated. In this paper, PAM-7 (Table 4) which contains more large-size pores (>19 μm: 14%) than other samples was
55 chosen as a scaffold to guide the fibroblast cellular growth. After the materials dehydrated using graded ethanol, only a few cells were found on the surface (Fig. 8), but there were plenty of cells inside especially in the larger pores (μm). In addition, the smaller pores in PAM-7 are about 60% in total, and these smaller pores
60 may be helpful to transport the nutrients into the whole scaffold for the cell growth by capillary action. When PAM-3 was used as a scaffold to foster the growth of fibroblast cells, only a small number of cells was found in the skeleton because the majority of the pores were smaller in size (small-size pores <10 μm: >82%;
65 large-size pores > 19 μm: <4%).

Conclusion

In this paper, a new amphiphilic cationic surfactant PVAc-*b*-PDMAEMA is synthesized by successive RAFT/ATRP polymerization. The results indicate that PVAc-*b*-PDMAEMA can
70 form the highly concentrated C/W emulsion (the internal phase

volume taking up 90% v/v). The emulsifying capacity of the surfactant is affected greatly by pH of solution and system pressure etc. PVAc-*b*-PDMAEMA is more suitable for use under acidic conditions. Comparing with some fluorinated ionic surfactants or non-ionic surfactants, PVAc-*b*-PDMAEMA can be a cheaper substitute for many applications. By the polymerization of the continuous phase of C/W emulsions, highly porous emulsion-templated materials with tunable pore-size and open-pores size are prepared. SEM analysis shows that the pore-size of these materials is mainly in the range of 1.5-50 μm . When the porous materials are used as scaffold to promote the growth of fibroblast cells, the cells are found to grow in the larger pores. The results show that these materials are suitable for tissue engineering. In future study, we plan to design porous materials with highly interconnected pore structure for a number of applications.

Acknowledgement

The authors are grateful to the Analysis and Test Center of Huazhong University of Science & Technology for characterizations. This work is financially supported by the Program for New Century Excellent Talents in University (NCET-10-0389), National Natural Science Foundation of China (50973037/51173058).

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