The preparation of poly(2-hydroxyethyl methacrylate) and poly{(2-hydroxyethyl methacrylate)-*co*-[poly(ethylene glycol) methyl ether methacrylate]} by photoinitiated polymerisation-induced phase separation in water

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Abstract

This paper describes the application of a photoinitiated polymerisation-induced phase separation method to the preparation of PHEMA and P[HEMA-*co*-(MeO-PEGMA)] hydrogels. PHEMA sponges having a morphology of agglomerated polymer droplets and interconnected pores were easily prepared from aqueous mixtures containing HEMA, EDGMA (crosslinker) and DPAP (photoinitiator). P[HEMA-*co*-(MeO-PEGMA)] copolymers having similar morphologies could also be prepared, provided that the proportion of MeO-PEGMA in the copolymer was relatively small. When higher proportions of MeO-PEGMA were used, the resulting polymers were gels rather than sponges, and did not show the sought after droplet/pore morphology. P[HEMA-*co*-(MeO-PEGMA)] copolymers having higher proportions of MeO-PEGMA and having a morphology of agglomerated polymer droplets and interconnected pores were easily prepared by addition of NaCl to the polymerisation mixture. Thus, incorporation of MeO-PEGMA and adddition of NaCl to the photopolymerisation mixtures provides an easy way of tuning the hydrophilicity of PHEMA copolymer sponges without compromising the desired porous morphology.

Keywords

PHEMA; photopolymerisation; porous hydrogel

1. Introduction

Hydrogels are three-dimensional polymeric networks in which hydrophilic macromolecular chains are chemically and/or physically crosslinked. Generally they are soft, flexible, have low surface friction, and can absorb and retain large amounts of water without dissolution. Their inherent hydrophilic nature renders them highly biocompatible [1, 2]. For these reasons, hydrogels represent a class of biomaterials well suited for use in biomedical applications involving the repair and replacement of soft tissues [1, 3].

Poly(2-hydroxyethyl methacrylate) (PHEMA) has received considerable attention as a biocompatible hydrogel. PHEMA and related copolymers have been used in a multitude of biomaterial applications, including soft contact lenses [4], artificial corneas [5], potential substrates for artificial skin [6], rhinoplasty surgery [7] and in drug delivery systems [8, 9]. The biocompatibility and hydrophilic nature of crosslinked PHEMA hydrogels provide a suitable platform for investigating potential scaffold materials to support tissue growth. Polymeric scaffolds used in tissue engineering applications generally require an open-pore morphology, in which the pores are larger than 10 μm in diameter [10-13], interconnected, and uniform throughout the material. This type of pore morphology has been proposed as the optimum to allow for cellular proliferation and tissue development [3, 13-15].

The porous structure of crosslinked PHEMA can be classified as either homogeneous or heterogeneous. Homogeneous hydrogels have a pore volume that is negligible relative to the volume occupied by the polymeric chains, range from non-porous to microporous (10 – 100 nm pores), and may be transparent to opalescent. Heterogeneous hydrogels will have a high volume of pores relative to polymer chains. Heterogeneous hydrogels are generally obtained via solution polymerisation and range in appearance from opalescent to opaque with pores ranging from 100 nm to 1 mm. Sponges are heterogeneous hydrogels where the pores are larger than 1 μm [16].

Formation of 3D morphological substructures suitable for polymeric scaffolds has previously been achieved via a variety of methods such as solvent casting and particulate leaching, melt moulding, freeze-drying techniques, membrane lamination, extrusion, electrospinning and gas foaming[17]. Generally, these methods are laborious and often

require the use of toxic reagents that may pose a risk when the final material is applied *in vitro* or *in vivo*. However, PHEMA hydrogel sponges that exhibit optimal pore morphology and allow for cellular penetration and tissue ingrowth can be prepared through a direct one-step polymerisation of HEMA in water [16]. The formation of these sponges by this one-step method has been described as polymerisation-induced phase separation and results in PHEMA sponges that display a characteristic agglomerated polymer droplet microstructure [13, 16, 18]. The suitability of the resulting sponges for biomaterials applications is exemplified in the development of a PHEMA artificial cornea, which consists of a non-porous transparent centre and a porous opaque outer annular skirt [5].

The water content in the initial polymerization mixture is the key parameter that controls the pore size and morphology of the resulting PHEMA hydrogels. Generally, an interconnected porous morphology is obtained from polymerization mixtures containing greater than 75 wt % water. PHEMA sponges have been prepared routinely by using either redox or thermal initiation [8, 9, 13, 16].

The aim of this work was to investigate alternative methods, in particular the use of photoinitiation, for the production of PHEMA and related copolymers having interconnected pore morphologies, and to improve their hydrophilicity for potential use as polymeric scaffolds in tissue engineering applications. The use of photoinitiated systems offers an attractive alternative over redox initiated systems as rapid curing times and relatively low curing temperatures are easily achieved [19]. Photopolymerisation is often the technique used to produce soft contact lenses from PHEMA [20]. Contact lenses call for optically transparent crosslinked PHEMA, which can be achieved either by bulk polymerisation or where the diluent (e.g. glycerine, ethylene glycol, water) [4, 21] does not exceed a critical concentration. Photopolymerisation techniques are also used in the preparation of hydrogels for controlled release systems [22]. This technique allows for the preparation of hydrogels in close to physiological conditions [19]. The studies that have explored photopolymerisation of HEMA together with a comonomer and water as the diluent, have been limited to using a concentration of water not exceeding 60 wt %, to obtain optically transparent homogeneous hydrogels [18, 22, 23]. We want to explore the use of photoinitiation as an alternative to producing heterogeneous hydrogels that possess an agglomerated-droplet morphology.

In addition to producing porous PHEMA homopolymers, we are aiming to fine-tune the hydrophilic nature of the hydrogels in order to obtain a range of materials suitable for use as polymeric scaffolds. The functionalisation of PHEMA chains with poly(ethylene glycol) (PEG) units would greatly alter the properties of resulting hydrogel. PEG is an extensively studied, generally biocompatible polymer that is very hydrophilic and possesses many properties that render it suitable for use as a biomaterial [24]. PEG features prominently as a bioconjugate in drug delivery systems, where it is covalently attached to proteins/peptides and/or pharmaceuticals in order to confer an increased solubility in biological systems as well as to render the resulting conjugates non-immunogenic [24].

In this paper we describe the preparation and characterisation of a range of PHEMA and PHEMA-PEG copolymers obtained by photoinitiated polymerisation-induced phase separation in water. The morphological and physical features of the hydrogels were characterised by scanning electron microscopy (SEM), thermal gravimetric analysis (TGA) and differential scanning calorimetry (DSC).

2. Materials and methods

2.1. Materials

2-Hydroxyethyl methacrylate (HEMA) (Bimax, Inc. USA, > 99.0 %) was distilled (b.p. 38 – 39 °C/0.1 mm Hg) and stored at -20 °C until use. Ethylene glycol dimethacrylate (EGDMA) (Polysciences, Inc.), tetraethylene glycol dimethacrylate (TEGDMA) (Fluka), 2,2-dimethoxy-2-phenylacetophenone (DPAP) (Irgacure 651, Aldrich, 97 %), sodium chloride (Fluka, AR grade) and deuterium oxide (ANSTO, Australia) were all used as received. Poly(ethylene glycol) methyl ether methacrylate (MeO-PEGMA) (Aldrich, M_n *ca.* 1100) was recrystallised from hot ether, to remove the inhibitor. All solvents were distilled prior to use. Deoxygenation of methanol and HEMA was achieved by at least three freeze-pump-thaw cycles. Water used for the preparation of polymers was obtained from a Millipore water purification system and was deoxygenated by exhaustive purging with nitrogen gas prior to use.

2.2. Monomer conversion studies via ^{1}H NMR spectroscopy

Monomer conversion with time was studied using time-course experiments carried out on a Bruker ARX-500 ^{1}H NMR spectrometer using a WATERGATE water suppression pulse programme. A typical procedure is described below (Entry A3, Table 1):
HEMA (120 μL, 130 mg, 1.0 mmol) was added via a gas-tight syringe to a solution of D_2O (65 μL) in water (515 μL) in an NMR tube sealed with a rubber septum under nitrogen. DPAP was added as a methanolic solution (5 μL of a 26 mg/mL DPAP solution). Care was taken to minimise exposure of the resulting solution to light. The NMR tube was suspended in the centre of a hollow quartz tube, which was positioned 10 cm from a UV lamp. The temperature of the NMR tube through out the experiment was maintained at 24 – 27 °C by a stream of compressed air passed through the hollow quartz tube. At one min intervals, the NMR sample was removed from the apparatus and the ^{1}H NMR spectra recorded until a conversion of >90 % of HEMA to PHEMA was achieved.

Table 1

Summary of experimental conditions and results obtained for the conversion studies of the photopolymerisation of HEMA

Entry	HEMA:H_2O[a]	DPAP (mol %)[b]	UV Exposure (min)	% Conversion[c]
A1	60:40	0.1	10	95
A2	20:80	0.01	30	92
A3	20:80	0.05	15	92
A4	20:80	0.1	15	94
A5	10:90	0.1	20	90

[a] The polymers are identified according to the weight ratio of water to HEMA used in the initial polymerisation mixture. Water contained 10 wt % D_2O.

[b] Relative to HEMA.

[c] % conversion was determined by monitoring the signal for the vinylic CH of HEMA relative to that of the methyl group of methanol (internal standard).

2.3. Hydrogel preparation

2.3.1. PHEMA hydrogels

Hydrogels were prepared in quartz vials via the photopolymerisation of HEMA in aqueous solutions according to the reagent formulations outlined in Table 2. The preparation of polymer B11 (Table 2) detailed below, is a typical procedure:

EGDMA (2 μL, 10.6 μmol) was added via syringe to a solution of HEMA (130 μL, 140 mg, 1.07 mmol) in water (560 μL) in a quartz vial under nitrogen. An ethanolic solution of DPAP (5 μL of a 57 mg/mL solution, 0.001 mmol) was then added, and the mixture was sonicated for approximately 1 min. The sample vial was then suspended in the centre of a hollow quartz tube positioned 10 cm from a UV lamp and irradiated for 20 min, whilst maintaining the temperature below 27 °C (using a stream of compressed air). The polymer was then carefully removed from the vial and immersed in water. The water was replaced with fresh water twice a day for a week to remove any unreacted HEMA monomer. Samples were stored under water at all times.

Table 2

Experimental details for the preparation of crosslinked PHEMA containing 1 mol % EGDMA.

Polymer	Water:HEMA[a]	DPAP(mol %)[b]	UV exposure (min)	Macroscopic appearance
B1	40:60	0.1	15	transparent
B2	50:50	0.1	15	transparent
B3	60:40	0.01	35	translucent
B4	60:40	0.05	20	translucent
B5	60:40	0.1	20	translucent
B6	70:30	0.1	20	white
B7	80:20	0.01	35	white
B8	80:20	0.01	35	white
B9	80:20	0.05	20	white
B10	80:20	0.1	20	white
B11	80:20	0.1	20	white
B12	80:20	0.5	15	white
B13	90:10	0.1	30	white
B14[c]	90:10	0.1	30	white

[a] The polymers are identified according to the weight ratio of water to HEMA used in the initial polymerisation mixture.

[b] Relative to HEMA monomer.

[c] After UV exposure, the polymer was cured at 50 °C for 24 h.

2.3.2. P[HEMA-co-(MeO-PEGMA)] hydrogels

The P[HEMA-*co*-(MeO-PEGMA)] copolymers were prepared according to the reagent compositions outlined in Table 3. The H_2O:HEMA ratio was kept constant at 80:20 w/w, and the photoinitiator and crosslinking agent (TEGDMA) were used at 0.1 mol % and 1 mol % respectively relative to HEMA. The amount of MeO-PEGMA was varied, as per Table 3. For samples where NaCl was used to promote phase separation, 0.8 M NaCl was used in place of water as the diluent in the polymerisation mixture.

Table 3

Experimental details for the preparation of P[HEMA-*co*-(MeO-PEGMA)] hydrogels

Diluent	Polymer[a]					
	80:20:1	80:20:2	80:20:4	80:20:6	80:20:7	80:20:8
H_2O	C1	C2	C3	C4	C5	
0.8 M NaCl		C6	C7		C8	C9

[a] The copolymers are identified based on weight ratio A:B:C, where A = part by weight water, B = part by weight HEMA, C = part by weight MeO-PEGMA.

2.4. Scanning electron microscopy (SEM)

Cross-sections (300 - 500 μm thick) of the hydrogels were obtained using a Vibratome 3000 instrument. Dehydration of the samples was achieved either by critical point drying (Emitech K850) or by freeze drying (Dynavac FD2) to constant mass. For critical point drying, the hydrated samples were soaked in acetone for at least 3 h. before being placed in the critical drying apparatus, where the samples were flushed three to four times with liquid CO_2 to remove the acetone and to ensure complete permeation of CO_2 liquid throughout the sample. The critical point was then reached by gradually increasing the temperature of the chamber to a value between 35 and 37 °C. A temperature above 31.1 °C was required to prevent the re-condensation of liquid CO_2.

Following dehydration, the samples were mounted on double-sided carbon tabs and coated with a 30 nm layer of carbon. The samples were imaged using a Zeiss 1555 VF-FESEM microscope at an accelerating voltage of 3kV, with working distance of 6 mm and an aperture of 10μm.

2.5. Thermogravimetric analysis (TGA)

TGA analysis was performed on the hydrated and dehydrated samples using a TA Instruments TGA Q50 thermoanalyser. The average mass of the samples for analysis was 8 ± 2 mg. The samples were heated in an aluminium pan at a rate of 10 °C/min to 550 °C under an atmosphere of argon (50 mL/min).

2.6. Differential scanning calorimetery (DSC)

The thermal glass transition temperatures of the copolymers were determined using a TA Instruments DSC Q10 Differential Scanning Calorimeter, at a heating rate of 10 °C/min from 0 °C to 180 °C, followed by an isothermal period for 5 min, and then cooling at 10 °C/min to 0 °C. The samples (2 to 4 mg) were ground into a powder before being sealed in aluminium pans.

3. Results and discussion

3.1. Homopolymerisation of HEMA

3.1.1. Monomer conversion studies

In order to be suitable for practical applications, the photopolymerisation of HEMA needs to achieve a high conversion of monomer to polymer. It was important to quantify the amount of time (UV exposure) required to reach an appropriate percentage conversion, and a monomer conversion of 90 % or higher was deemed a realistic target for the photopolymerisation reactions. The extent of photopolymerisation of HEMA in water could be readily monitored using ^{1}H NMR spectroscopy, by observing the decrease in intensity of the vinylic signals of HEMA relative to the intensity of signals due to an internal standard (methanol). Thus, using the photoinitiator DPAP, a series of experiments was carried out to investigate the effect of the initiator concentration (Fig. 1) and the water concentration (Fig. 2) on monomer conversion.

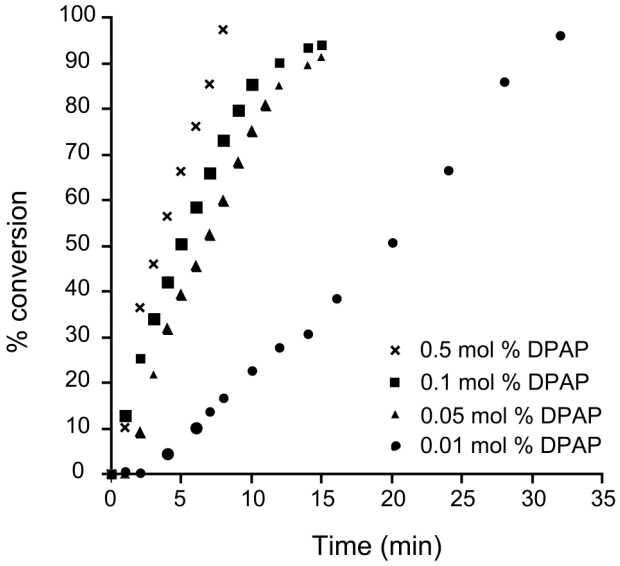


Fig. 1 Conversion plot for the polymerisation of HEMA in water (water:HEMA 80:20 w/w) in the presence of various quantities of DPAP

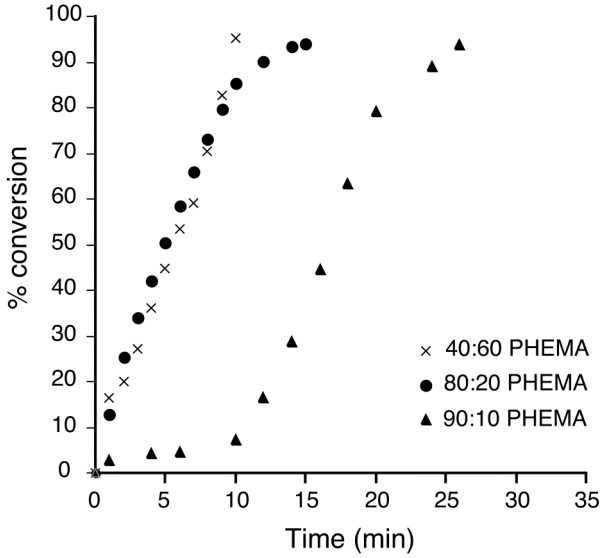


Fig. 2 Conversion plot for the polymerisation of HEMA in the presence of 0.1 mol % DPAP at various water to HEMA ratios.

The effect of the photoinitiator concentration on the percentage monomer conversion as a function of time is illustrated in Fig. 1. From the conversion curve, it can be seen that a high monomer conversion (> 90 %) was reached within 30 min for the range of DPAP concentrations examined. Above 0.05 mol % DPAP, less than 15 min of UV irradiation was required. Monomer conversions of greater than 90 % were also achieved within a period of 20 min of UV irradiation for the range of water to HEMA ratios studied. Fig. 2 shows that the

conversion curves are approximately linear for polymerisation of 80:20 and 40:60 w/w water:HEMA mixtures.[1] In the case of 90:10 w/w mixtures, an initial lag period of 10 min was observed, followed by an almost linear conversion curve. These results indicate that a high conversion of monomer to polymer (> 90 %) could be achieved within relatively short periods of time (i.e. within 30 min) across a range of experimental conditions commonly employed during the preparation of both homogeneous (40:60 PHEMA) and heterogeneous (80:20 PHEMA) PHEMA hydrogels. These results are consistent with previous work involving water as a diluent [20, 21]. Thus, it does not appear that phase separation (which results in a visually opaque hydrogel) is a significant hurdle for photoinitiated polymerisation of HEMA.

3.1.2. Variation of the water content and its effect on polymer morphology

The proportion of water in the initial polymerisation mixture is considered one of the most important variables in the formation and morphology of PHEMA hydrogels [9, 13, 16, 25]. Therefore a series of crosslinked PHEMA polymers were prepared by varying the water:HEMA ratio in the initial polymerisation mixture from 40:60 to 90:10 whilst maintaining constant crosslinker (EGDMA, 1.0 mol %) and photoinitiator (0.1 mol %) concentrations. Furthermore, to compare the efficacy of photoinitiation relative to redox-initiation, the experimental conditions used in this study were maintained as close as possible to those for redox-initiated polymerisations reported in the literature [8, 9, 13, 16]. The morphology and porosity of the resulting crosslinked polymer samples were examined by SEM. Both conventional SEM [16] and variable pressure (VP) SEM [8, 13, 26] have been used successfully by others to characterise the surface and internal morphological features of PHEMA hydrogels. Conventional SEM techniques require the complete dehydration of a specimen prior to imaging and this dehydration can been achieved by air-drying [27], freeze-drying [28], critical point drying, [16] or freeze-fracture etching [9]. A potential consequence of any drying process are artefacts due to irreversible alteration of the structure (size and/or

[1] The polymers are identified according to the ratio of weights of water to HEMA used in the initial polymerisation mixture. For example, an 80:20 PHEMA sample is prepared from a mixture containing 80 parts water and 20 parts HEMA.

shape of the hydrogel, at both macroscopic and microscopic dimensions). In an effort to avoid any ambiguities with respect to interpreting SEM images, all samples were prepared for imaging using two methods – critical point drying and freeze drying. Unless otherwise stated, all of the SEM images shown in this work are from samples that were prepared by critical point drying. As the internal morphology of the hydrogels is of utmost importance in this study, and thus only cross-sections of the polymer samples were imaged.

The SEM images of the photoinitiated PHEMA hydrogels (with varying water content) showed significant changes in polymer morphology with increasing water content in the polymerisation mixture (Fig. 3). It is important to note that all PHEMA homopolymers (B1 to B14, Table 2) could be readily dehydrated by both critical point drying and freeze drying techniques. The SEM images of these materials displayed size, shape and microstructural features that were indistinguishable regardless of the method used for sample preparation. The hydrogels prepared using low water concentrations (i.e. 40:60 PHEMA and 50:50 PHEMA, samples B1 and B2 respectively, Table 2) were optically transparent. SEM images of these materials displayed smooth internal surfaces devoid of any apparent porosity (images not shown). SEM images of PHEMA samples prepared using higher proportions of water in the polymerisation mixture, namely the PHEMA hydrogels with H_2O:HEMA ratios of 60:40, 70:30, 80:20 and 90:10 (Fig. 3), revealed a general increase in their porosity. Whilst the 60:40 hydrogels (Fig. 3A) can be described as essentially non-porous (consistent with previous results on similar systems [16]), the 80:20 hydrogels (Fig. 3C) and 90:10 hydrogels (Fig. 3D) displayed a well-defined polymer droplet morphology (droplet diameters *ca.* 3 μm and 1 μm respectively) and void spaces ranging from 10 to 20 μm in diameter. The transition from a discontinuous to continuous porosity occurred between the 70:30 and 80:20 polymers (Fig. 3B and 3C). This result agrees with previous findings that this crucial transition occurred when 75 wt % water was used in the polymerisation mixture and EGDMA (0.7 mol %) was used as the crosslinking agent. [16] The observed morphologies of the hydrogels produced via the photoinitiation system are consistent with redox or thermally initiated systems of analogous hydrogels.[16, 18]

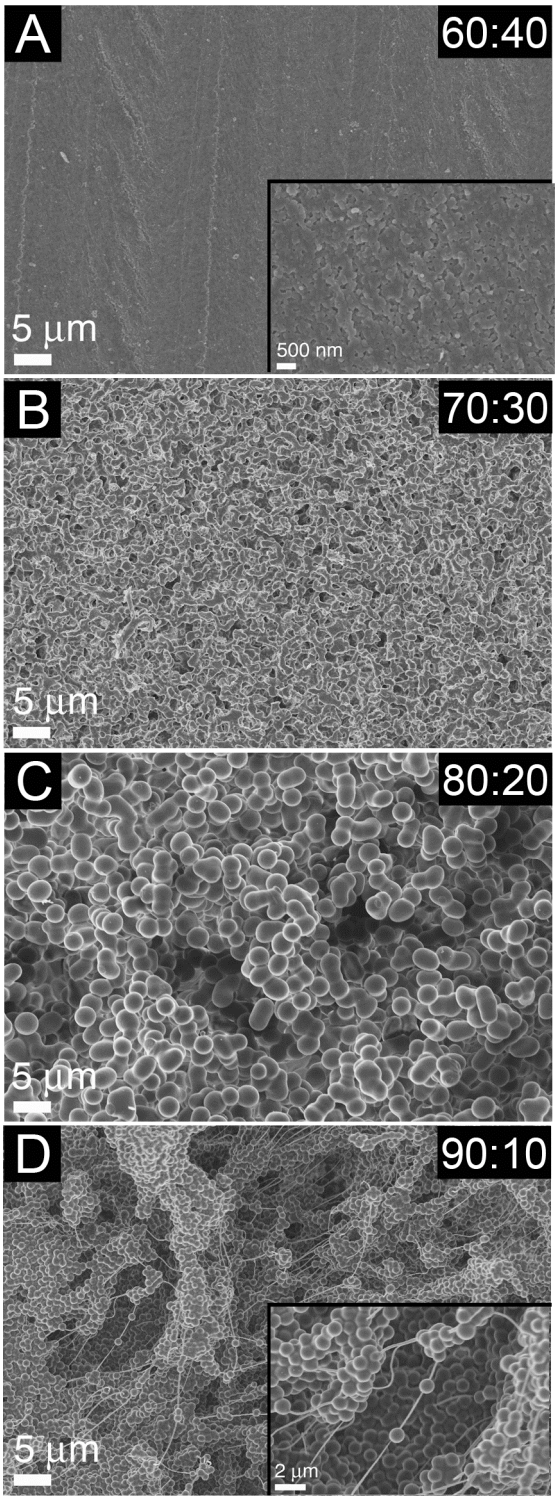


Fig. 3 SEM images of PHEMA hydrogels produced from mixtures containing the water:HEMA ratios indicated, EGDMA (1.0 mol % relative to HEMA) and DPAP (0.1 mol % relative to HEMA). **A:** 60:40 (B5, Table 2). **B:** 70:30 (B6, Table 2). **C:** 80:20 (B11, Table 2). **D:** 90:10 (B13, Table 2).

The reduced droplet size seen in the images of the 90:10 hydrogel compared to those for the 80:20 hydrogel may be attributed to the high monomer dilution in the 90:10

polymerisation mixture, which would result in an early onset of phase separation and thus a smaller droplet size [8, 25]. In addition, the 90:10 hydrogel displayed poor mechanical properties and could not be easily manipulated. In an attempt to improve the sample's strength, a heat treatment procedure in which the sample was incubated at 50 °C for 24 h was attempted, and the resulting cured polymer was analysed by SEM and compared to its uncured analogue (Fig. 4). From the SEM images, it can be seen that heat treatment resulted in the apparent annealing of the polymer droplets into a highly ordered array of spheres together with a partial loss of porosity. This change coincided with a considerable increase in mechanical strength.

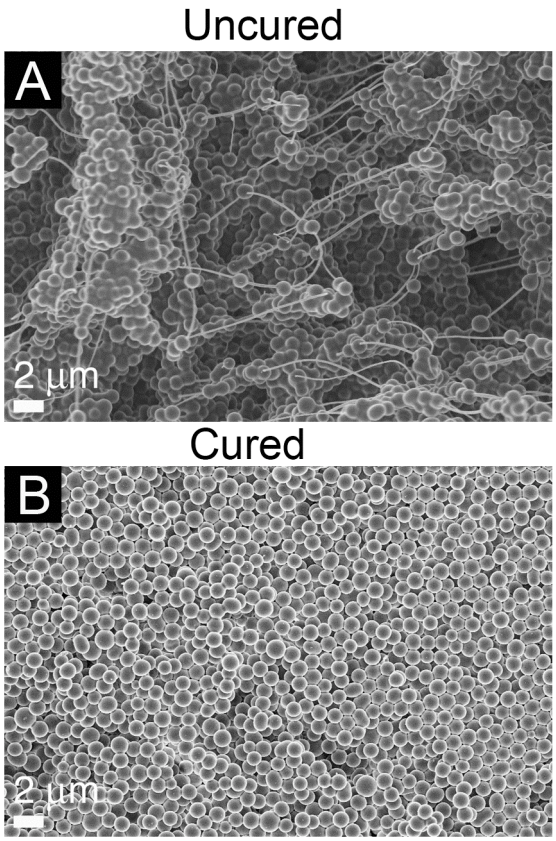


Fig. 4 SEM images of an uncured vs cured 90:10 PHEMA hydrogel. **A:** Uncured (B13, Table 2). **B:** Cured (B14, Table 2).

3.1.3. Effect of initiator concentration on hydrogel morphology

Previous studies using redox or thermally initiated systems [16] indicated that 60:40 PHEMA hydrogels could be produced with the desired interconnected porous morphology, provided that the concentration of the initiator and/or crosslinking agent were considerably elevated. This effect manifested as a change in the morphological features of the 60:40

PHEMA polymers from homogeneous to heterogeneous (agglomerated droplets, pore size approximately 10 μm in diameter) after a 25-fold increase in redox initiator concentration [16]. A study of the effect of very high concentrations of the photoinitiator in the present study was not possible due to the low solubility of DPAP in the aqueous polymerisation mixtures, but increasing the concentration of DPAP by a factor of 10 had no significant influence on morphology of either the 60:40 or 80:20 hydrogels (Fig. 5).

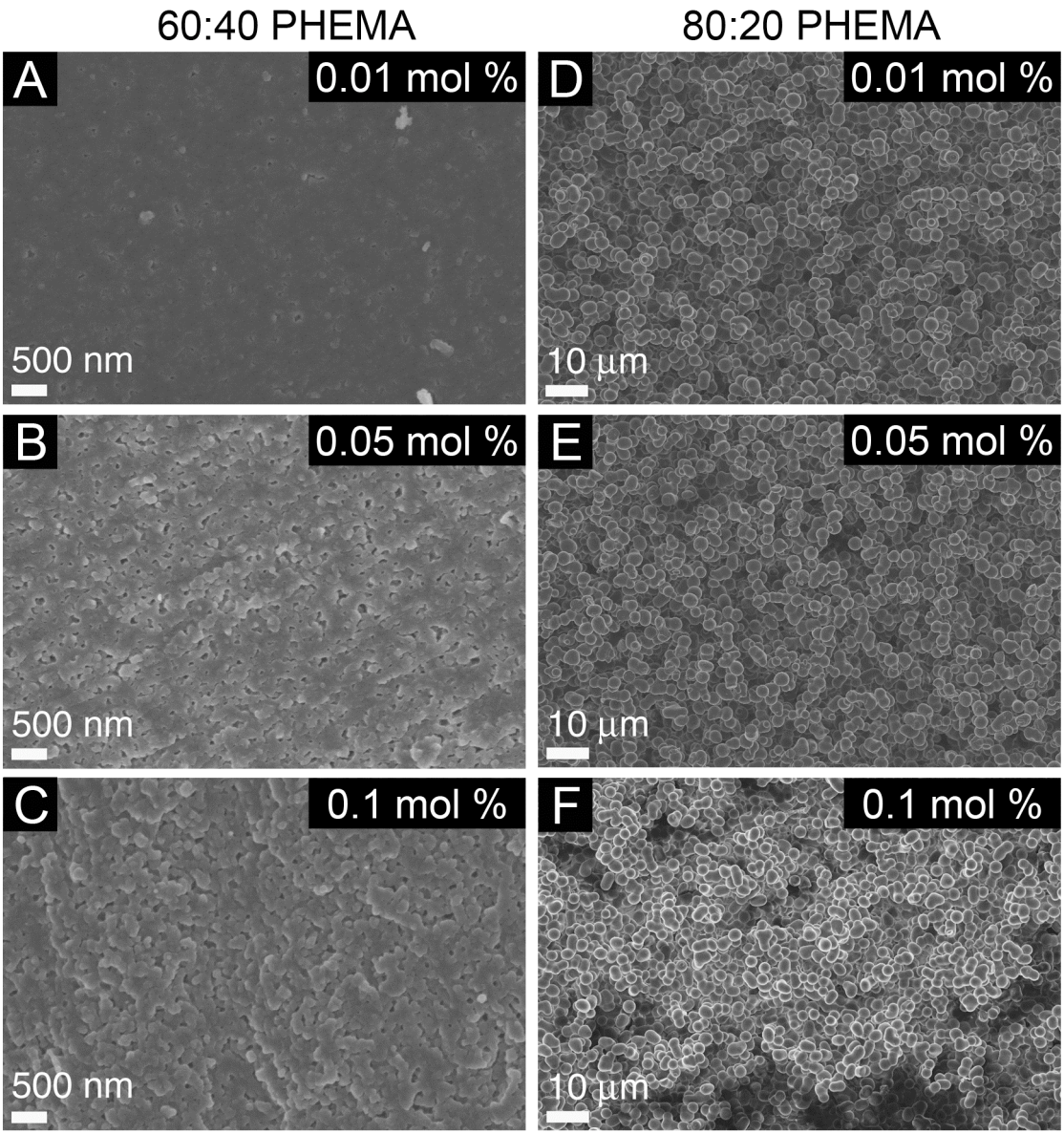


Fig. 5 SEM images of 60:40 PHEMA hydrogels (A, B, C) and 80:20 PHEMA hydrogels (D, E, F) formed using the concentrations of DPAP indicated. **A:** 0.01 mol % (B3, Table 2). **B**: 0.05 mol % (B4, Table 2). **C**: 0.1 mol % (B5, Table 2). **D**: 0.01 mol % (B8, Table 2). **E**: 0.05 mol % (B9, Table 2). **F**: 0.1 mol % (B11, Table 2).

3.2. Copolymerisation of HEMA with MeO-PEGMA

3.2.1. Preparation of P[HEMA-co-(MeO-PEGMA)] hydrogels

The P[HEMA-*co*-(MeO-PEGMA)] hydrogels were prepared and then analysed *via* conventional SEM. The 80:20:1 and 80:20:2 copolymers were dehydrated without apparent damage *via* critical point drying. Samples of materials containing higher proportions of MeO-PEGMA, however, shrank to about 10% of their initial size during critical point drying. This result suggested that the microstructure of the materials had collapsed, a conclusion that was supported by the appearance of SEM images of the shrunken samples, which revealed featureless surfaces devoid of pores (images not shown). The effect of MeO-PEGMA content on the behaviour of materials during critical point drying is presumably a consequence of changes in glass transition temperature (T_g) with increasing MeO-PEGMA content. T_g for crosslinked PHEMA and P[HEMA-*co*-(MeO-PEGMA)] samples was determined by DSC. T_g for PHEMA was found to be 119 °C, which is consistent with previous reports [29, 30]. The presence of PEG structural units dramatically lowers the T_g. Thus, T_g values for 80:20:2 and 80:20:4 P[HEMA-*co*-(MeO-PEGMA)] polymers (Fig. 6) were determined to be 46 °C and 36 °C respectively. During the critical point drying process, samples were heated to approximately 30 - 40 °C. PHEMA and 80:20:2 P[HEMA-*co*-(MeO-PEGMA)] have T_g values above this range, and thus withstand the critical point drying process. For 80:20:4 P[HEMA-*co*-(MeO-PEGMA)] and other copolymers that have higher proportions of MeO-PEGMA, however, the drying temperature exceeds T_g, resulting in the sample becoming more gel/rubber-like, and a collapse of the microstructure of the sample during the critical point drying process.

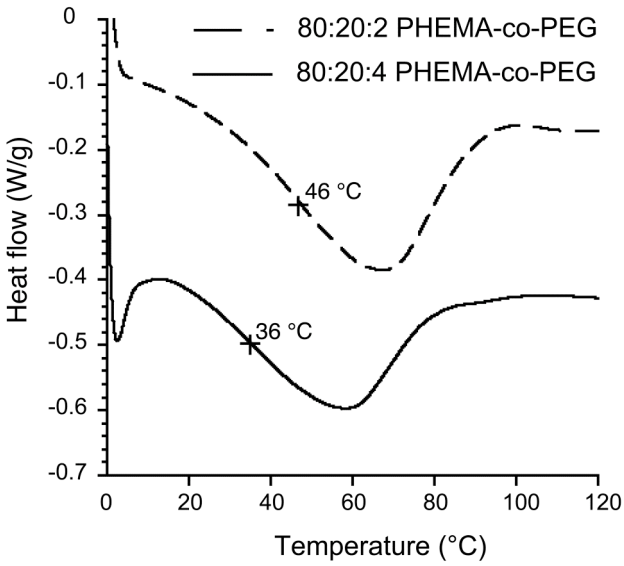


Fig 6 DSC for 80:20:2 and 80:20:4 P[HEMA-*co*-(MeO-PEGMA)] hydrogels.

As discussed above, for PHEMA homopolymers there was no apparent difference between samples dried by critical point drying or freeze drying. Compared to critical point drying, however, freeze drying is generally considered an inferior method of dehydration due to surface tension effects leading to specimen collapse [31, 32]. Fig. 7 shows the SEM images of the 80:20:2 hydrogel dehydrated by (A) critical point drying and (B) freeze drying. Whilst the sample prepared by critical point drying maintains the characteristic polymer droplet morphology, the freeze dried sample displays large openings and there is noticeable coalescence of polymer droplets. This result suggests that freeze drying is not an ideal method for preserving the microstructure during the dehydration of PHEMA hydrogels containing PEG moieties. Despite this difficulty, freeze drying proved to be less destructive to P[HEMA-*co*-(MeO-PEGMA)] containing high proportions of MeO-PEGMA—such samples maintained their macroscopic size and shape during freeze drying. Therefore, since the samples containing higher proportions MeO-PEGMA completely collapsed during critical point drying process, it was felt that for these samples, freeze drying, although not an ideal technique, would provide a reasonable compromise to allow some information on polymer morphology to be collected.

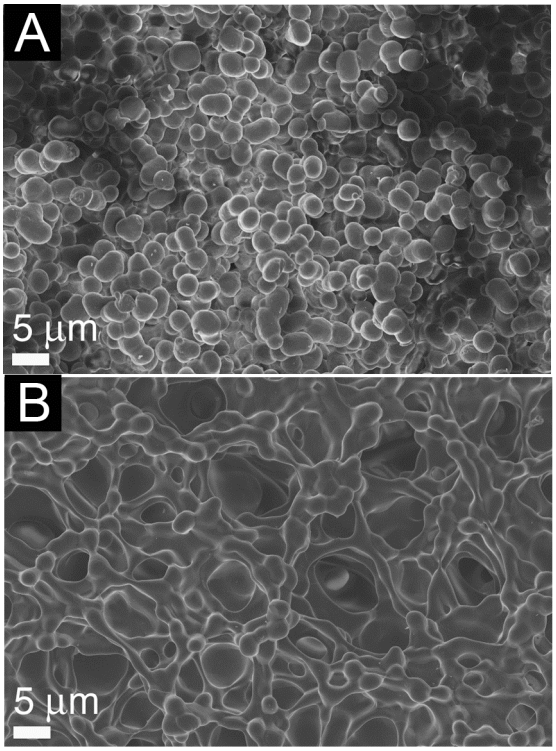


Fig. 7 SEM images of 80:20:2 4 P[HEMA-*co*-(MeO-PEGMA)] hydrogels produced with 1.0 mol % TEGDMA and 0.1 mol % DPAP (C2, Table 3). Samples prepared by: **A**: critical point drying; and **B**: freeze drying.

The 80:20:1 and 80:20:2 hydrogels were visually opaque materials. The SEM images of the critical point-dried 80:20:1 and 80:20:2 hydrogels (Fig. 8A & B respectively) clearly show polymer droplet conglomerates, with pores of diameters of the order of 10-20 μm. The morphology displayed by these materials is consistent with a polymerisation-induced phase separation mechanism being responsible for their formation.

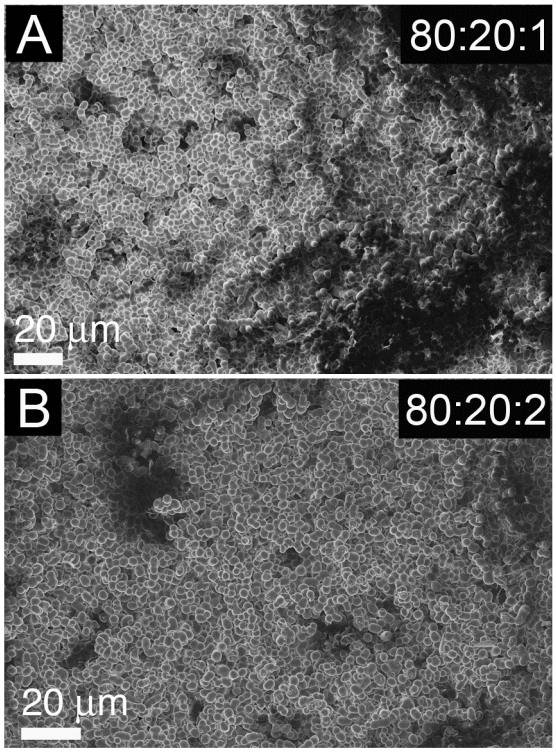


Fig. 8 SEM images of P[HEMA-*co*-(MeO-PEGMA)] hydrogels produced with 1.0 mol % TEGDMA and 0.1 mol % DPAP and various proportions of MeO-PEGMA. Samples were prepared for SEM by critical point drying. **A**: 80:20:1 (C1, Table 3). **B**: 80:20:2 (C2, Table 3).

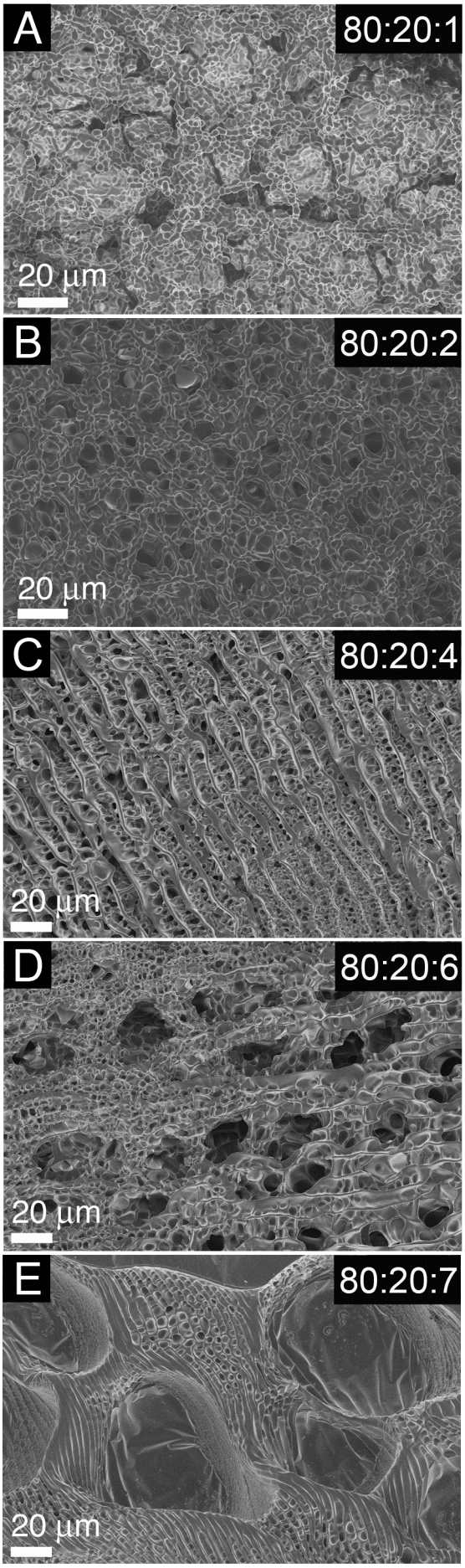
Fig. 9 shows SEM images of the P[HEMA-*co*-(MeO-PEGMA)] hydrogels that were prepared for imaging by freeze drying. The SEM image of the 80:20:1 hydrogel (Fig. 9A) displays a polymer droplet morphology, albeit somewhat distorted when compared to its critical point-dried counterpart (Fig. 8A). Similarly, the SEM image of the 80:20:2 hydrogel shows a distorted polymer droplet morphology throughout the freeze-dried sample (Fig. 9B).

Samples prepared from polymerisation mixtures containing higher proportions of MeO-PEGMA (i.e. 80:20:4, 80:20:6 and 80:20:7 samples, Fig. 9C to E) were visually transparent hydrogels and displayed a discontinuous porosity with a general increase of the pore size from 5 μm (80:20:4) to 85 μm (80:20:7). In addition, the 80:20:7 polymers exhibited a "dual porosity" where small pores (approximately 5 μm) were present in the walls of the larger pores (approximately 60 to 85 μm) (Fig. 9E). A similar effect has been previously observed in PHEMA hydrogels that were grafted with a sulfonated PEG macromonomer [33]. In conclusion, the SEM images of the P[HEMA-*co*-(MeO-PEGMA)] hydrogels indicate that the transition from a porous sponge to a non-porous gel occurs

between the copolymers of compositions 80:20:2 (Fig. 9B) and 80:20:4 (Fig. 9C), a result that is consistent with the macroscopic appearance of the samples.

Fig. 9 SEM images of P[HEMA-*co*-(MeO-PEGMA)] hydrogels produced using various proportions of MeO-PEGMA, TEDGMA (1.0 mol % relative to HEMA) and DPAP (0.1 mol % relative to HEMA). Samples were prepared by freeze drying. **A**: 80:20:1 (C1, Table 3). **B**: 80:20:2 (C2, Table 3). **C**: 80:20:4 (C3, Table 3). **D**: 80:20:6 (C4, Table 3). **E**: 80:20:7 (C5, Table 3).

(**Fig. 9** provided on following page)


A
80:20:1
20 µm
B
80:20:2
20 µm
C
80:20:4
20 µm
D
80:20:6
20 µm
E
80:20:7
20 µm

To account for the appearance and microscopic morphology of the samples, it is necessary to consider the mechanism of phase separation polymerisation, which is governed by the thermodynamic interactions between the solvent/diluent and the growing polymer chains of the polymer network. The introduction of a more hydrophilic comonomer to the PHEMA backbone (i.e. MeO-PEG groups from MeO-PEGMA) would increase the solubility of the growing polymer chains in the solvent/diluent (water) and thus should act to suppress phase separation. This is exemplified by the appearance (both macro- and microscopic) of the copolymer networks prepared with increasing proportions of MeO-PEGMA. Polymerisations involving lower proportions of MeO-PEGMA resulted in opaque polymers that have the distinct agglomerated droplet morphology typically present in heterogeneous hydrogel sponges. Thus, for these samples, polymerisation did indeed induce phase separation, which suggests that the addition of MeO-PEGMA in relatively low concentrations does not significantly alter the insoluble nature of the growing PHEMA network. However, polymerisations involving higher proportions of MeO-PEGMA resulted in translucent to clear gels that did not display the agglomerated polymer droplet morphology, indicating that the polymerisation did not induce phase separation. The growing polymer chains remain soluble throughout the polymerisation process, suppressing polymer precipitation and preventing the phase separation process from occurring. The increase in hydrophilic nature of the P[HEMA-*co*-(MeO-PEGMA)] polymers with increasing MeO-PEG content is further substantiated by the thermal gravimetric analysis (TGA) of the hydrated polymers (Fig. 10). TGA of the fully hydrated hydrogels indicated that all of the samples underwent a significant mass loss at temperatures lower than 100 °C, consistent with the loss of water during the heating cycle. Samples having higher proportions of MeO-PEGMA showed larger mass losses in this temperature region, consistent with higher water contents.

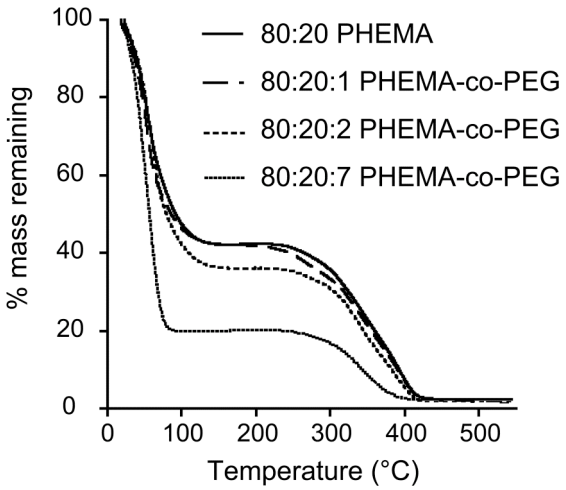


Fig. 10 TGA of hydrated PHEMA homopolymer and P[HEMA-*co*-(MeO-PEGMA)] copolymers.

3.2.2. Polymerisation in the presence of NaCl

Although P[HEMA-*co*-(MeO-PEGMA)] copolymers containing relatively high proportions of MeO-PEGMA (C3-C5, Table 3; Fig. 9C-9E) possess enhanced hydrophilicity, their lack of interconnected pores would make them unsuitable templates for tissue engineering applications. Our work indicates that the homogeneity of the samples containing high proportions of MeO-PEGMA results from suppression of phase separation during crosslinking polymerisation. Thus, a means of inducing phase separation for these more hydrophilic polymers was needed. The addition of NaCl, or "salting-out", is a common method for promoting the phase separation of a material from water. This method works because the addition of NaCl to an aqueous mixture acts to enhance the ionic strength of the mixture, causing less-polar constituents to form a separate phase. This method has been used previously to promote polymerisation-induced phase separation in the preparation of PHEMA sponges[18] and microspheres [34].

All the polymerisation mixtures having monomer ratios that led to transparent homogeneous P[HEMA-*co*-(MeO-PEGMA)] hydrogels could be made to form spongy materials with condensed droplet morphology by the addition of NaCl to the pre-polymer mixture (C6 to C9, Table 3). To demonstrate this point, Fig. 11 shows SEM images of 80:20:4 copolymers prepared with either water or 0.8 M NaCl as diluent.

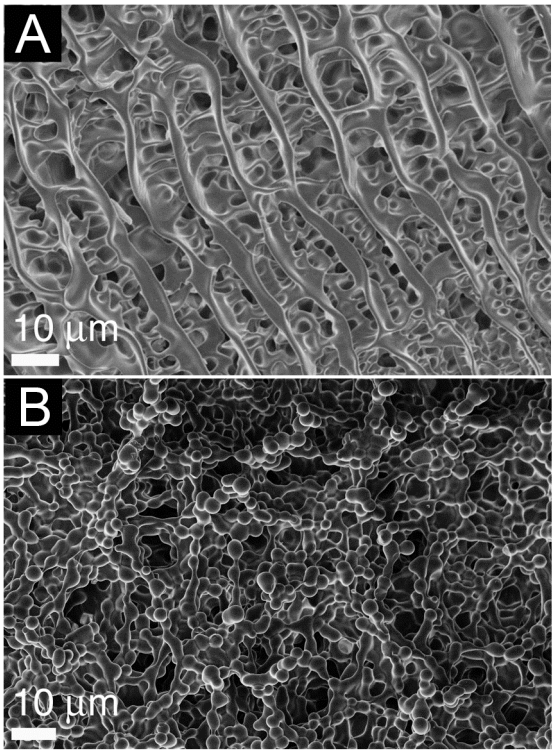


Fig. 11 SEM images of 80:20:4 P[HEMA-*co*-(MeO-PEGMA)] hydrogels produced using as diluent: (**A**) water (C3, Table 3); and (**B**) 0.8 M NaCl (C7, Table 3). The hydrogels were prepared with 1 mol % TEGDMA and 0.1 mol % DPAP relative to HEMA.

Interestingly, the P[HEMA-*co*-(MeO-PEGMA)] samples prepared using 0.8 M NaCl as diluent were more robust and could better withstand both critical point and freeze drying processes than similar samples prepared with water as the diluent. Whereas 80:20:4, 80:20:6, and 80:20:7 P[HEMA-*co*-(MeO-PEGMA)] samples prepared without NaCl shrank substantially during critical point drying (see above), similar samples prepared in the presence of NaCl maintained their shape during the critical point drying process, and their SEM images showed the expected polymer droplet morphology (Fig. 12A-D). SEM images of samples of 80:20:2 P[HEMA-*co*-(MeO-PEGMA)] prepared in the presence of NaCl were similar (*cf.* Fig. 12A, E) regardless of the drying method used. As the proportion of MeO-PEGMA in the samples increased, however, there was a progressive deterioration in polymer microscopic morphology as seen in SEM images of the freeze dried samples (*cf.* Fig. 12B-D and F-H).

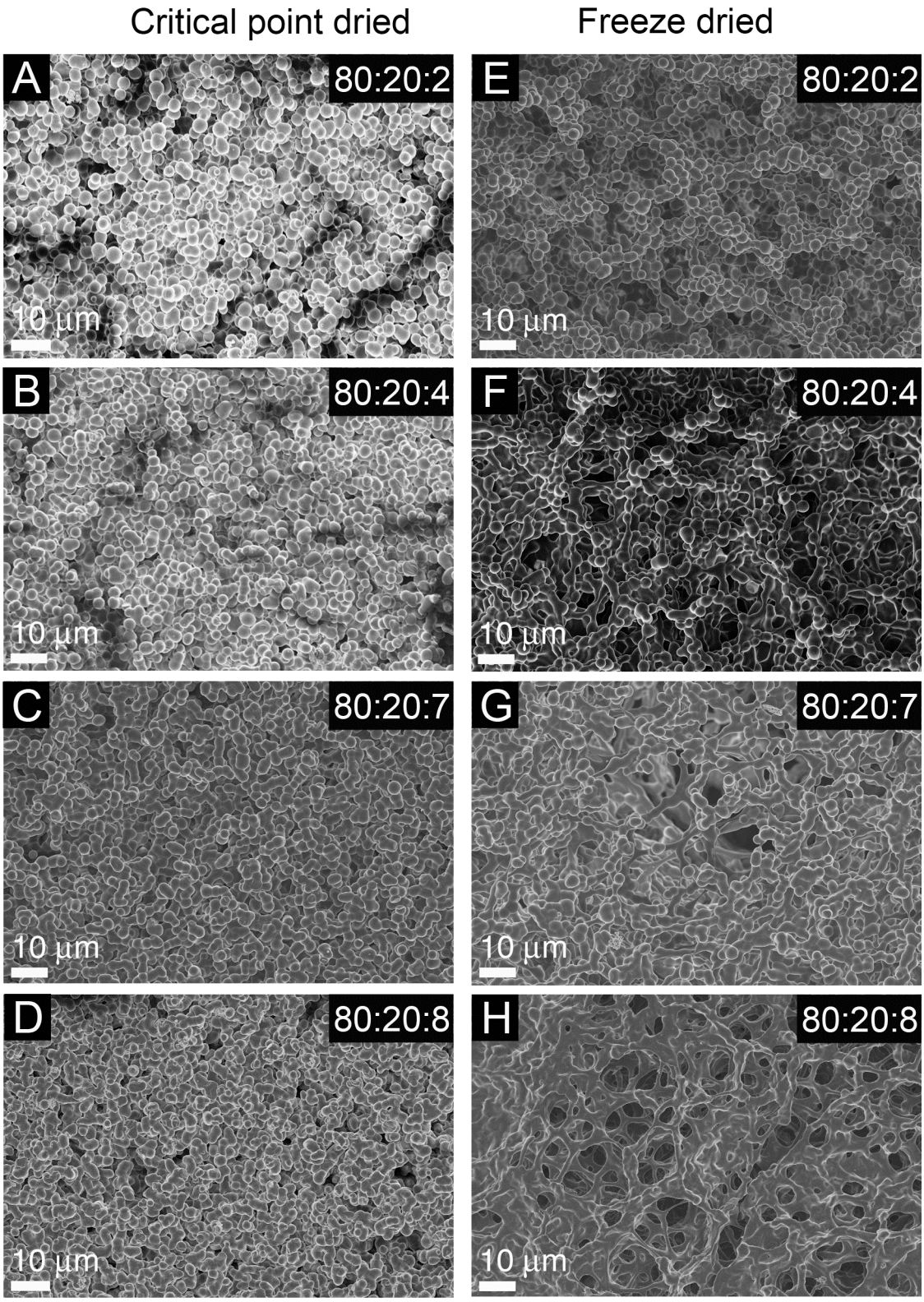


Fig. 12: SEM images of P[HEMA-*co*-(MeO-PEGMA)] hydrogels produced using 0.8 M NaCl as diluent, with 1.0 mol % TEGDMA and increasing proportions of MeO-PEGMA: **A** and **E**, 80:20:2 (0.8 M NaCl:HEMA:PEG) (C6, Table 3); **B** and **F**, 80:20:4 (C7, Table 3); **C** and **G**, 80:20:7 (C8, Table 3); **D** and **H**, 80:20:8 (C9, Table 3)

5. Conclusions

PHEMA and P[HEMA-*co*-(MeO-PEGMA)] hydrogels having a morphology of agglomerated polymer droplets were prepared by a photoinitiated polymerisation-induced phase separation method from aqueous mixtures. In copolymerisations using higher proportions of MeO-PEGMA (HEMA:MeO-PEGMA < 0.1), the P[HEMA-*co*-(MeO-PEGMA)] materials were gels rather than sponges, and did not show the sought after droplet/pore morphology. These copolymerisations yielded materials with the polymer droplet morphology when NaCl was included in the polymerisation mixture. Thus, incorporation of MeO-PEGMA and addition of NaCl to the photopolymerisation mixtures provides an easy way of tuning the hydrophilicity of PHEMA copolymer sponges without compromising the desired porous morphology.

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