Video Article

Microbubble Fabrication of Concave-porosity PDMS Beads

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Abstract

Microbubble fabrication (by use of a fine emulsion) provides a means of increasing the surface-area-to-volume (SAV) ratio of polymer materials, which is particularly useful for separations applications. Porous polydimethylsiloxane (PDMS) beads can be produced by heat-curing such an emulsion, allowing the interface between the aqueous and aliphatic phases to mold the morphology of the polymer. In the procedures described here, both polymer and crosslinker (triethoxysilane) are sonicated together in a cold-bath sonicator. Following a period of cross-linking, emulsions are added dropwise to a hot surfactant solution, allowing the aqueous phase of the emulsion to separate, and forming porous polymer beads. We demonstrate that this method can be tuned, and the SAV ratio optimized, by adjusting the electrolyte content of the aqueous phase in the emulsion. Beads produced in this way are imaged with scanning electron microscopy, and representative SAV ratios are determined using Brunauer–Emmett–Teller (BET) analysis. Considerable variability with the electrolyte identity is observed, but the general trend is consistent: there is a maximum in SAV obtained at a specific concentration, after which porosity decreases markedly.

Video Link

The video component of this article can be found at <http://www.jove.com/video/53440/>

Introduction

Polydimethylsiloxane (PDMS) is one of the most widely used silicone compounds. Its biocompatibility has led to widespread use in implant and other biomedical engineering structures^{1,2}. It is trivially cross-linked into elastic structures using an organosilyl compound (such as triethoxysilane), a simple and reliable procedure which has made it useful for cast polymer applications where some flexibility is required³. Once cross-linked, PDMS is largely inert, particularly in biological conditions, and is therefore useful for a variety of food and medical applications^{4,5}. Ease of casting, chemical inertness, and hydrophobicity have made it a natural choice for microfluidic devices^{6,7}. Its affinity for non-halogenated, non-polar organic compounds has made it a popular stationary phase in separations chemistry⁸⁻¹⁰.

Recently, microbubble fabrications have been used to generate porous beads for use as catalyst structural substrates or in chemical separations^{11,12}. In both applications, ideal materials will have a maximized surface-area-to-volume (SAV) ratio for best efficiency. In a microbubble fabrication process, microstructuring of materials is typically accomplished by isolating the polymer in aliphatic “microbubbles” by emulsification in an aqueous continuous phase. The initial report of microporous PDMS beads produced them by mechanical emulsification of two phases (aliphatic and aqueous)¹³. The stock PDMS liquid (and its cross-linking agent) is dissolved into the aliphatic phase, which is structured into microscopic beads by being forced to cavitate within the (continuous) aqueous phase. The emulsification is stabilized by the addition of a non-ionic surfactant. When the emulsion is added dropwise to a heated bath, solid beads form by agglomeration of the microbubbles into clusters of tiny spheres of cross-linked PDMS. Our goal in this protocol is to modify this procedure to develop beads with an inverted porosity to improve the SAV ratio of the material.

As reported previously, control of the beads can be directed to some extent by the aliphatic:aqueous ratios in the emulsion. However, we have reported recently that addition of platinum(IV) chloride (PtCl₄) inverts the porosity: materials are formed in which the PDMS is riddled with concave pores¹⁴. This indicates that the aqueous layer cavitates inside the aliphatic one, despite having similar aliphatic:aqueous ratios to those published in the original work¹³. The primary advantage of our method is that this concave porosity should naturally result in an increased SAV ratio, and thus, improved efficiency for analytical chemistry applications. While we are continuing to explore the specific effects of the addition of the platinum compound, we show here that the same effect can be accomplished using any aqueously soluble ionic compound, though perhaps to a reduced extent. Because our techniques also differ in some key aspects from what has been previously reported, we present our protocols here as a video to encourage others to extend our methods. Most notably, we use a common bath sonicator of the type used to clean glassware or other equipment, rather than the (considerably more expensive) probe sonicator often used in microbubble fabrication. This adjusted approach to the microbubble fabrication procedure could potentially be extended for the production of large quantities of bulk materials as well, creating porous sheets or slabs which could have applications for biomedical devices, aerospace and automotive industry, or substrates for chemical catalysis. Users seeking to generate high-SAV-ratio, microstructured materials using other similar polymers for such analyses may find that our protocols can be extended to any polymer for which the microbubble emulsion technique can be applied.

Protocol

1. Preparation of Emulsion

1. Emulsion Contents

1. Mass an appropriate amount of salt to produce 10 ml of 0.03-M solution. For platinum(IV) chloride measure 0.101 g, for zinc(II) chloride (ZnCl_2) measure 0.032 g, and sodium chloride (NaCl) measure 0.018 g.
2. In individual test tubes, dissolve each salt into 10 ml of DI water. Set aside for later use.
3. Use a 20-ml, sealable glass vial for the contents throughout this procedure. Tare a balance to the glass vial.
4. Weigh vinyl-terminated polydimethylsiloxane by slowly pouring it over a stir rod and into the glass vial resting on the zeroed scale. Weigh out 1.02 g (equivalent to 1.080 ml).
NOTE: The high viscosity of this polymer makes pipetting impractical.
5. Pipette 1.02 ml of n-Heptane to the vial. Add 2 drops of non-ionic surfactant (sorbitan monoleate) to the vial. Pipette 0.3 ml of salt solution and 0.45 ml of DI-water to the vial.
6. Seal the glass vial by screwing the lid on tightly. Shake vigorously for 60 sec to initiate the emulsion before beginning sonication.

2. Construction of Water-bath Sonicator Apparatus

1. Fill sonicator with water up to the minimum fill line. Add 250 ml of tap water to a 400-ml beaker. Fill the 400-ml beaker with ice so that the water level is just at the rim.
2. Place this beaker inside the water-bath sonicator. Check the fill-line on the sonicator, adjusting if needed. Place a ring stand directly beside the water-bath sonicator.
3. Using two ring stand clamps, position them so that an arm is extended out, perpendicular to the ring stand and another one is extended towards the water bath so that it is pointing downwards into the beaker filled with ice water. Attach another clamp to the ring stand with a thermometer down in the 400-ml beaker so that the temperature can be monitored throughout sonication.

3. Emulsification Procedure

1. Place the emulsion-containing vial so that it is fully submerged in the 400-ml beaker by securing it into the clamp protruding down into the beaker.
2. Ensure that the glass vial containing the PDMS mixture is not touching the sides of the beaker to eliminate heat caused by friction.
3. Turn on the sonicator and set sonication time for 7 min. Because the emulsion is very heat sensitive, ensure that the temperature inside the beaker is between 0 and 5 °C throughout sonication. Start sonication once the temperature inside the beaker is desirable.
4. After 7 min of sonication, remove the vial and gently shake/swirl for 1 min, holding the top of the vial to eliminate any clumps that might form in the emulsion.
5. Dispose of the contents of the beaker. Refill with 250 ml of water and add ice to the fill to within 1 cm the top of the beaker.
6. Place the vial back in the clamp. Submerge it under the ice water to resume sonication for 7 min.
7. Repeat steps 1.3.3 to 1.3.6 for a total of eight, 7-min sonication periods, or until the sonication appears to be homogenous and no clumps are present. Store at RT.
NOTE: The emulsification should be stable for several days, but can be recovered by sonication as above in 7-min intervals until it appears homogenous. Store at RT.

2. Cross-linking

1. Setup for Addition of Triethoxysilane/Surfactant Solution

1. Pipette 5.4 ml triethoxysilane into a test tube. Place in a test tube rack under the hood for later use.
2. Fill a 400-ml beaker with ice water and place it under the hood. Next to the 400-ml beaker place a ring stand with a clamp attached, extended directly over the opening of the beaker. This will be the ice-bath for the addition of triethoxysilane.
3. Place a hot plate on the other side of the ring stand. Fill an 800-ml beaker with approximately 700 ml of tap water. Place it on the hot plate.
4. Turn on the hot plate and maintain a temperature of 75 to 85 °C inside the 800-ml beaker. Attach a clamp with a thermometer to the ring stand so the temperature of the water inside the 800-ml beaker can be monitored.
5. Produce the surfactant solution by dissolving 0.5 g of sodium dodecyl sulfate to 375 ml of water (4.62 mM). Add approximately 10 ml of surfactant solution to a clean, empty test tube.
6. Attach another clamp to the ring stand under the hood with the surfactant test tube secured such that its liquid level is below the surface of the water inside the 800-ml beaker. Allow 10 min for thermal equilibration.
7. Wet a piece of filter paper and place it in the top of a small funnel. Place the stem of the funnel inside a 250-ml Erlenmeyer flask and place the flask under the hood.

2. Addition of Triethoxysilane

1. Place the emulsion vial in the clamp over the ice-water beaker inside the hood. Position the PDMS mixture in the clamp so that the vial contents are below the surface of the water. The addition of triethoxysilane causes an exothermic reaction, so the emulsion must be kept cold in order to maintain its structure.
2. Remove the lid from the glass vial to avoid gaseous build up.
3. Slowly pour the test tube containing triethoxysilane into the glass vial in a continuous stream over a period of approximately 10 sec (roughly 0.5 ml/sec).

CAUTION: The addition of triethoxysilane initiates an exothermic reaction and the release of caustic hydrogen chloride (HCl) gas. The vial will become extremely hot and a toxic gas will evolve from the mixture. DO NOT STIR the contents while adding the triethoxysilane.

4. After adding triethoxysilane completely, gently stir the contents with a glass stir rod while wearing a heat protective glove. Wait for 2 min or until gas stops evolving from the vial.
 5. Following cross-linking, there is no phase separation visible in the sample. If clumps are present, seal the vial and shake rigorously for 20 sec while holding the vial by the lid.
3. Bead production
1. Use a clean, glass Pasteur pipette to draw the cross-linked emulsion from the glass vial. Add the cross-linked emulsion drop-wise to the surfactant solution (which should be maintained between 75 and 85 °C) into the test tube taking as little time as possible in between drops.
 2. 30 sec to 1 min after the addition of the emulsion, the surfactant solution will slowly begin to evolve gas as solids begin to form inside the test tube.
 3. While wearing heat protective gloves, take the test tube out of the clamp and pour its entire contents into the filtration apparatus under the hood. Filter for 5 min. Remove the filter paper from the filter.
 4. Transfer the filtered solids onto a watch glass and separate beads for O/N drying under the hood. Cleaning of the beads can be postponed indefinitely. Store dried beads at RT in a sealed glass vial until needed, and clean immediately prior to use.
4. Bead Cleaning
1. Create another filtration apparatus under the hood and place the dried beads inside the funnel on top of the filter paper.
 2. Use a plastic wash bottle filled with DI-water to rinse the beads gently, moving them around slightly to ensure all the beads are rinsed.
 3. Let the beads dry for 1 hr by placing them on a watch glass under the hood. Use a wash bottle filled with hexanes to rinse the beads using the same method for rinsing with water.
 4. Place the beads on a watch glass. Place the watch glass and the beads under the hood to dry.
 5. After the beads are completely dry, place them in a small sealable glass vial and store at RT for future use.
5. Mounting the Beads for SEM Analysis and SEM Settings
1. Place a strip of double-sided carbon conductive tape on top of the stub onto which the beads will be mounted. Using scissors, trim around the stub to ensure no tape hangs over the edges.
 2. Place a piece of filter paper under the stub on a flat surface. Remove the top layer from the tape so that the adhesive underside is exposed.
 3. Gently pour the beads over the stub. Some beads will stick to the tape, but most will bounce off and land on the filter paper. Pour these back into the vial if they stayed on the filter paper. Repeat if necessary, washing any beads (according to 2.4.2 to 2.4.5) that become contaminated.
 4. To ensure the beads are secure on the stub, use a bulb syringe and lightly blow very closely to the stub surface. Pour more beads over the stub if only a few adhered to the tape. Ensure that all beads are secure before placing the stub into the SEM chamber and evacuating it.
 5. Once the samples have been mounted properly they are now ready to undergo SEM analysis¹⁵. Collect images in LOW VAC mode at 15 keV to optimize the resolution of the bead's surface features.

Representative Results

Representative SEM images of beads arising from emulsions with different electrolyte conditions are shown in **Figure 1**. **Figure 1A** shows a bead similar to those obtained by DuFaud, *et al.*¹³, produced using our procedures, without the addition of any electrolyte. Beads shown in **Figure 1B-D**, resulting in different morphologies for each metal ion. For all images shown, 300 µl of 0.03-M electrolyte solutions were used in place of 300 µl of the DI water for the aqueous phase, giving an electrolyte concentration of 0.012 M in that phase. Higher resolution images are shown in **Figure 2** of beads produced with no electrolyte (a) and with ZnCl₂.

Brunauer–Emmett–Teller (BET) analysis^{16,17} of the beads was performed using nitrogen isotherms at five different pressures. The beads shown in **Figure 1** were taken from the same batch of materials used for the BET analysis in each case. The BET analysis yields quantitative values for surface area to volume ratio, listed in **Table 1**.

Figure 1. SEM Images of entire beads. SEM images of PDMS beads produced by the microbubble fabrication technique described here. Beads produced without the addition of any electrolyte to the aqueous layer (A) are exclusively convex porosity. Length scales for all images are given by the scale bar on the figure. Those produced with a net concentration of 0.012 M metal concentration for PtCl_4 (B), ZnCl_2 (C), and NaCl (D) show different morphologies, including the substantial addition of concave pores due to microbubble formation, as indicated in the circled area. [Please click here to view a larger version of this figure.](#)

Figure 2. Detailed SEM Images. Closer SEM images of (A) beads produced without electrolyte added, and (B) with ZnCl_2 . Without the addition of electrolyte, spherical substructures are generally larger and more closely packed than with the addition of ZnCl_2 , which contributes significantly to the increase in surface-area-to-volume ratio. Length scales for all images are given by the scale bar on the figure. [Please click here to view a larger version of this figure.](#)

Salt added	BET SAV Ratio (cm^2/cm^3)	SAV Improvement (relative to control)
None (control)	361.6	1
PtCl_4	1,849	5.1
ZnCl_2	11,060	30.6
NaCl	298.9	0.83

Table 1. BET Analysis. Surface-area-to-volume (SAV) ratios, determined by Brunauer–Emmett–Teller (BET) isotherm analysis, of the materials produced using aqueous phases with 0.012-M aqueous solutions of different electrolytes. First column letters indicate the corresponding image panel in **Figure 1**. SAV ratios are based on the total surface area per unit mass, the mass of the sample, and the total cold free space of the sample.

Discussion

The beads produced using this protocol (and by adjusting the electrolyte concentration and identity) are fundamentally different from those produced with a low-ionic strength emulsion, as seen by comparison of **Figure 1A** to the other SEM images in **Figure 1**. Our initial report used PtCl_4 with the intention of further catalyzing the polymerization cross-linking at the aqueous-aliphatic interface¹⁴. In that report, large concave indentations were seen. Since that report, we have refined our procedures to optimize for SAV ratio. Without washing the beads, elemental analysis of domains within the SEM images indicate that platinum was found almost exclusively within the concave pores which are found throughout the bead structure. Because this platinum was easily removed by agitating the beads in water, we concluded in our original report that the platinum was simply residue on the PDMS surface, rather than being incorporated into the polymer. This implied a catalytic role for the

platinum in the curing process. The beads shown in **Figure 1** are characterized by the appearance of spherical substructures, largely convex, but those produced with added electrolyte also contain concave spherical regions such as the circled portion of **Figure 1B**. The presence of electrolyte appears to reduce the size of the microbubbles in the emulsion. Detailed images suggest that, based on the sizes of the spherical substructures, the bubbles in the emulsion are on the order of 0.2 μm to 2 μm in diameter with ZnCl_2 , but are more commonly 1-10 μm in diameter without electrolyte (consistent with previous work by DuFaud, *et al.*¹³).

The data presented here suggest that cheaper, more earth-abundant metals can be used instead of the original PtCl_4 : concave porosity beads are produced regardless of the identity of the metal ion used, though to significantly varying degrees. Use of ZnCl_2 provides a drastic increase in the SAV ratio, six times as high as for PtCl_4 , and approximately 30 times as high as for the control procedure, which contains no electrolyte. The BET results are in excellent agreement with the SEM images: for ZnCl_2 , numerous concave pockets, a few microns in diameter, are seen distributed throughout the structure. The detailed images in **Figure 2B** also demonstrate that there is a considerably more internal space when beads are produced in the presence of ZnCl_2 . Both of these features sites serve to increase the available surface area for adsorption, a feature which is highly desirable for separations where the PDMS is used as a stationary phase.

For all electrolytes tested to date, there is an optimum concentration of salt, one that maximizes the surface porosity of the beads produced, and it appears to be a total concentration of 0.012 M, regardless of the metal counterion. Below and above this optimum concentration, the SAV ratio is less than the maximum, and appears to decrease monotonically from the maximum in either direction. This dependence on salt concentration, rather than being based solely on the presence of a catalytically-active metal ion in solution, suggests that the effect of electrolyte on microbubble fabrication is to change the properties of the emulsion. Without added salt content, the emulsion is best described as cavities of aliphatic (*i.e.*, polymer-containing) phase imbedded into a more continuous aqueous phase. When salt is added, the surface tension is altered in such a way that the aliphatic phase is continuous in some places, with dispersed aqueous bubbles. When the emulsion is suddenly heated in the surfactant bath, the drop retains this structure as the PDMS cures. This results in beads which have less-spherical shapes, but are imbedded with holes due to the presence of aqueous pockets, even for metal chlorides of sodium, which would not be expected to have a substantial catalytic effect. Although any water-soluble salt should work in principle to accomplish this effect, and we note that there is minimal dependence of the optimum concentration on metal ion identity, there is a distinct advantage to metals known to catalyze organic carbon-carbon coupling reactions. This suggests that, while the change in ionic strength is needed to cause the phase inversion, the primary determining factor in adjusting the SAV ratio is the ability of the metal to act as a catalyst for the cross-linking of individual polymer strands, directing it to occur preferentially at the aqueous/aliphatic interface.

The most critical aspect of our protocol is that the sonication process and the addition of triethoxysilane can both generate large amounts of heat if not controlled carefully. This heat can cause solids to form prematurely, which will make the generation of the desired beads impossible. The sonication problem has been largely addressed by the use of a bath-type sonicator, and the inclusion of ice in the bath to help regulate this temperature. The 7 min sonication periods were found to be optimal in reducing unwanted clumping of the emulsion, in part because after approximately 7 min of sonication, most of the ice in the bath has melted. Addition of triethoxysilane must be done with active stirring and with the mixture submerged in an ice bath.

Compared to the original report producing convex porosity PDMS beads, our protocol demonstrates a significant advantage in the SAV ratio of the product. We have demonstrated that the addition of even inexpensive salts to the aqueous phase of an emulsion used in microbubble fabrication of porous polymer beads can lead to drastic changes in the morphology of the end material. While the presence of any salt appears to result in an emulsion in which the aliphatic phase can become continuous, only by the addition of catalytically-active metal ions is the SAV ratio increased by a factor of 30. Although we have not tested this protocol on any other polymer, we expect that any polymer which is cross-linked and heat-cured (limitations of our protocol) could be used with this process to generate concave-porosity microstructured beads. In such an extension, it is likely that the specific electrolyte concentrations will need to be optimized for the emulsion being used to ensure that the aliphatic phase is continuous, and the aqueous phase is discrete.

Disclosures

The authors have nothing to disclose.

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