

A Thermal Expulsion Approach to Homogeneous Large-Volume Methacrylate Monolith Preparation; Enabling Large-scale Rapid Purification of Biomolecules

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ABSTRACT: Numerous efforts have been dedicated to the synthesis of large-volume methacrylate monoliths for large-scale biomolecules purification but most were obstructed by the enormous release of exotherms during preparation, thereby introducing structural heterogeneity in the monolith pore system. A significant radial temperature gradient develops along the monolith thickness, reaching a terminal temperature that supersedes the maximum temperature required for structurally homogeneous monoliths preparation. The enormous heat build-up is perceived to encompass the heat associated with initiator decomposition and the heat released from free radical-monomer and monomer-monomer interactions. The heat resulting from the initiator decomposition was expelled along with some gaseous fumes before commencing polymerization in a gradual addition fashion. Characteristics

of 80 mL monolith prepared using this technique was compared with that of a similar monolith synthesized in a bulk polymerization mode. An extra similarity in the radial temperature profiles was observed for the monolith synthesized via the heat expulsion technique. A maximum radial temperature gradient of only 4.3°C was recorded at the center and 2.1°C at the monolith peripheral for the combined heat expulsion and gradual addition technique. The comparable radial temperature distributions obtained birthed identical pore size distributions at different radial points along the monolith thickness. © 2008 Wiley Periodicals, Inc. *J Appl Polym Sci* 109: 2426–2433, 2008

Key words: macroporous polymer; large-volume; exothermic; polymerization; temperature

INTRODUCTION

Methacrylate monoliths synthesized from the polymerization of ethylene glycol dimethacrylate and glycidyl methacrylate in the presence of a porogen and an initiator were introduced a decade ago for the purification of biomolecules.¹ Since then, many papers have described the numerous applications of these monoliths on the analytical scale^{1–6} while only a few have actually considered the scale-up possibilities for large-scale applications.^{7,8} The synthesis of large-volume homogeneous methacrylate monolithic polymer is a complex process. This is mainly because of the large amount of exotherms associated with the polymerization process; causing pronounced temperature deviations that significantly affects the structural homogeneity of the monolith.⁹

Also, the unstirred nature of the polymerization within the mold results in a limited capacity to uniformly distribute the heat of polymerization. This causes an overall radial temperature deviation from the desired polymerization temperature and radial variation of exotherm magnitude across the content of the mold. As demonstrated by Viklund et al.¹⁰ any significant variations in temperature within the mold is expected to generate a monolith with a non-uniform pore structure. These are the obvious reasons why most of the works reported to date are focused on the development of small-sized monoliths. Control of polymerization temperature and excellent heat transfer capacities are the key parameters required for the preparation of large monoliths with reproducible and homogeneous pore and surface structures.^{9,11} Podgornik et al.¹¹ prepared several annular monoliths with different outer diameters and then embedded one into another. By this technique, the reactant mixture is reduced and consequently the temperature distribution is effectively controlled. Nevertheless, it can be quite complicated to prepare large-volume monoliths, because there is a high probability of damaging the pieces during the embedding process. There is also the likely occurrence of large dead volumes in the final monolith art

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piece. Peters et al.⁹ proposed that during the preparation of large-volume monoliths, gradual addition of the feedstock could control the polymerization rates so as to decrease the temperature gradient in the mold. This method minimized the extent of reaction exotherm and resulted in the production of monoliths with improved uniformity in pore structure. To further improve the pore structure uniformity, we incorporate a heat expulsion step prior to the polymerization to significantly reduce exothermicity; thus we report herein a heat expulsion technique for the synthesis of large-volume methacrylate monolith with a uniform pore structure and compare its characteristics with that of a similar monolith synthesized in a bulk polymerization mode. The effect of radial temperature gradient on the pore properties of the monolith is also reported.

THE HEAT EXPULSION TECHNIQUE

As already established, the critical dependency of the pore structure of methacrylate monoliths on temperature shifts due to exotherms endorses the complexities involved in the synthesis of large-volume methacrylate monoliths. The concept of heat expulsion emanates from the view that the extreme heat build-up encompasses the heat associated with initiator decomposition and the heat released from free radical–monomer and monomer–monomer interactions. As a result, expulsion of a portion of the heat of decomposition of the initiator as well its accompanying fumes prior to polymerization will minimize the amount of heat build-up during the polymerization. By this technique, the polymerization will commence straightaway with the free radical (resulting from the initiator decomposition) with minimal heat build up. On the basis of the aforementioned concept, we wish to derive a mathematical proof based on heat balances to predict the effect of the heat expulsion step on the temperature profile within the mold for a cylindrical monolith since monoliths with cylindrical geometry relatively exhibits a lower pressure drops.^{12–14} The mathematical modeling of the temperature profile during polymerization in a closed mold is quite complex. The main problem is the flow by convection inside the mold caused by radial temperature gradients during the polymerization. The convective flow enhances heat transfer that influences the radial temperature profile and the polymerization rate. It is recognized that these convective flows are not easy to predict even in simple systems where changes in physical properties are negligible.¹⁵ We however describe the combined heat transfer as only conduction, where the effective thermal conductivity (k_{eff}) integrates the convective part via the product of Nusselt number (Nu) and

thermal conductivity (k). Nusselt number approaches unity for a perfect conductive transfer system. The polymerization mold consists of a very thin glass column. The thin nature of the glass column wall allows negligible heat transfer resistance. It is also chemically inert to the polymerization feedstock. The glass column is immersed in a thermostated water bath at a constant temperature while receiving free radical/porogen and monomer mixtures to commence polymerization after a thermal equilibrium has been established with the water bath. Considering the geometry of the system based on the above-mentioned simplifications, the steady-state heat transfer equation in cylindrical coordinates can be written as;

$$\frac{\partial T}{\partial t} = \frac{\sigma_f}{r} \cdot \frac{\partial}{\partial r} \left(r \frac{\partial T}{\partial r} \right) + \frac{\Delta H_{\text{TL}}}{\rho C_p} = 0 \quad (1)$$

where σ_f is the effective thermal diffusivity in m^2/s , ΔH_{TL} is the total heat released per unit volume of the polymerization feedstock in W/m^3 , ρ is the density of the polymerization feedstock in kg/m^3 and C_p is the specific heat capacity of the feedstock in $\text{J}/\text{kg K}$. Rearranging eq. (1) gives;

$$-\frac{\Delta H_{\text{TL}}}{\rho C_p \sigma_f} r = -\frac{\Delta H_{\text{TL}}}{k} r = \frac{\partial}{\partial r} \left(r \frac{\partial T}{\partial r} \right) \quad (2)$$

where $k = \rho C_p \sigma_f$ is the thermal conductivity of the monomer mixture in W/mK .

The system is simplified as in eq. (2) with the assumptions that the heat released per unit volume (ΔH_{TL}) is constant during the polymerization reaction and is homogeneously released over the entire volume. It is also assumed that the thermal conductivity (k) is constant and that the entire system is at thermal equilibrium. Both ΔH_{TL} and k are dependent on the polymerization rate, which is in turn dependent on the temperature distribution inside the mold. However, the heat expulsion technique employed minimizes extreme temperature deviations from the polymerization temperature, thus making the assumptions worthwhile. Integrating eq. (2) and taking into account boundary conditions gives the solution below:

$$-\frac{\Delta H_{\text{TL}}}{2k} r^2 + \xi_1 = r \frac{\partial T}{\partial r} \quad (3)$$

where ξ_1 is the constant of integration. Evaluation of ξ_1 with boundary conditions is as shown below;

At the center of the cylindrical mold where

$$r = 0, \quad \frac{\partial T}{\partial r} = 0 \Rightarrow \xi_1 = 0$$

Therefore,

$$-\frac{\Delta H_{TL}}{2k} r^2 = r \frac{\partial T}{\partial r} \quad (4)$$

Integrating eq. (4) and taking into account boundary conditions gives the solution:

$$-\frac{\Delta H_{TL}}{4k} r^2 + \xi_2 = T \quad (5)$$

where ξ_2 is the constant of integration. Evaluation of ξ_2 with boundary conditions is as shown below;

At the inner wall of the cylindrical mold where

$$r = r_0, \quad T = T_0 \Rightarrow \xi_2 = T_0 + \frac{\Delta H_{TL}}{4k} r_0^2$$

Therefore,

$$T = \frac{\Delta H_{TL}}{4k} (r_0^2 - r^2) + T_0 \quad (6)$$

where T_0 and r_0 are the inner wall temperature and radius of the mold respectively. Since we wish to find the maximum temperature (T_{max}) inside the mold, we ought to find first the radial position at which the maximum temperature occurs (r_{max}). At the maximum temperature, $dT/dr = 0$ and $r = r_{max}$. Differentiating eq. (6) and applying the maximum temperature conditions gives;

$$\left(\frac{dT}{dr}\right)_{r=r_{max}} = -\frac{\Delta H_{TL}}{2k} r_{max} = 0 \Rightarrow r_{max} = 0$$

Therefore,

$$T_{max} = \frac{\Delta H_{TL}}{2k} r_0^2 + T_0 \quad (7)$$

Disintegrating the total heat released per unit volume of polymerization feedstock (ΔH_{TL}) into the heat associated with initiator decomposition (ΔH_i) and the heat associated with the interaction of active species such as monomer–monomer and free radical–monomer interactions (ΔH_{sp}) gives;

$$T_{max} = \left(\frac{\Delta H_{sp}}{2k} r_0^2 + T_0\right) + \frac{\Delta H_i}{2k} r_0^2 \quad (8)$$

Thus, expelling ΔH_i ($\Delta H_i \rightarrow 0$) results in a maximum temperature $T'_{max} < T_{max}$ and minimizes the radial temperature gradient that would otherwise be experienced for a bulk polymerization system. The minimization of the radial temperature gradient along the length of the monolith guarantees homogeneity in the pore size distribution of the entire monolith since remarkable shifts in temperature distorts the uniformity of the pore structure of the monolith.

EXPERIMENTAL

Chemicals/reagents

Ethylene glycol dimethacrylate (EDMA) (M_w 198.22, 98%), glycidyl methacrylate (GMA) (M_w 142.15, 97%), cyclohexanol (M_w 100.16, 99%), azobisisobutyronitrile (AIBN) (M_w 164.21, 98%) and methanol (HPLC grade, M_w 32.04, 99.93%) were purchased from Sigma-Aldrich.

Bulk synthesis of large-volume methacrylate monolith

The polymerization feedstock was prepared by reacting 60% : 40% v/v GMA as the functional monomer and EDMA as the crosslinker respectively, and the resulting mixture combined with cyclohexanol as the porogen in the ratio 40% : 60% v/v; making a solution with total volume 80 mL. AIBN (1% w/w with respect to monomer) was used to initiate polymerization. The polymer mixture was sonicated for 20 min and sparged with N_2 gas to expel dissolved O_2 gas. The polymerization mold was placed in a water bath for 18 h at 60°C. Radial temperature profiles within the mold were monitored by inserting thermocouples (Pyrosales, NSW, Australia) directly into the polymerization mixture at three radial points; center, 6 and 12 mm positions. The polymer was washed to remove all porogens and other soluble matters with methanol in a soxhlet extractor for 24 h and dried in an oven at 70°C (MEMMERT, Model 400, Germany).

Heat expulsion approach to large-volume methacrylate monolith synthesis

Figure 1 shows schematically the technique adopted for the monolith synthesis by heat expulsion. Cyclohexanol/AIBN mixture (P+I) and the monomer mixture (MM) were preheated to a temperature $T_0 = 50^\circ\text{C}$ and isothermally pumped (P1 and P2) simultaneously at flow rates of 0.10 and 0.07 mL/min, respectively, into the polymerization mold ($20 \times 2.5 \text{ cm}^2$ glass column) which is immersed in a water bath at

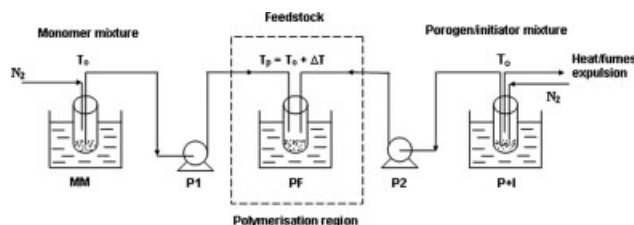


Figure 1 A schematic representation of the large-volume methacrylate polymer synthesis methodology via heat expulsion and gradual addition. MM, P1, PF, P2, and P+I represent monomer mixture, pump 1, polymerization feedstock, pump 2, and porogen/initiator mixture respectively.

$T_p = 60^\circ\text{C}$. The heat/fumes resulting from the AIBN decomposition was expelled prior to the isothermal pumping. In another instance, the cyclohexanol/porogen mixture was discharged immediately into the monomer mixture after the heat/fumes has been expelled to commence polymerization. Radial temperature profiles within the mold were monitored by inserting thermocouples (Pyrosales, NSW, Australia) directly into the polymerization mixture at three radial points; center, 6 and 12 mm positions. The polymerization feedstock compositions and conditions were identical to that of the bulk polymerization.

Characterization of the polymer pore properties

The pore structure of the polymer in the dry state was studied by mercury intrusion porosimetry at different radial positions along the length of the polymer using a micrometrics mercury porosimeter (Autopore III, USA). Three pieces of monolith sample sliced from the different radial positions of the polymer were placed on a sticky carbon foil that was attached to a standard aluminum specimen stub. The samples were vapor deposited with gold using a sputter coater (DynaVac, model SC 150, Australia). Microscopic analyses of the samples were carried out using a high resolution field emission scanning electron microscope (JEOL JSM-6300F, Japan) at a voltage of 15 kV.

RESULTS AND DISCUSSION

Bulk synthesis of methacrylate monolith

The aforementioned complexity in synthesizing homogeneous large-volume methacrylate monolith

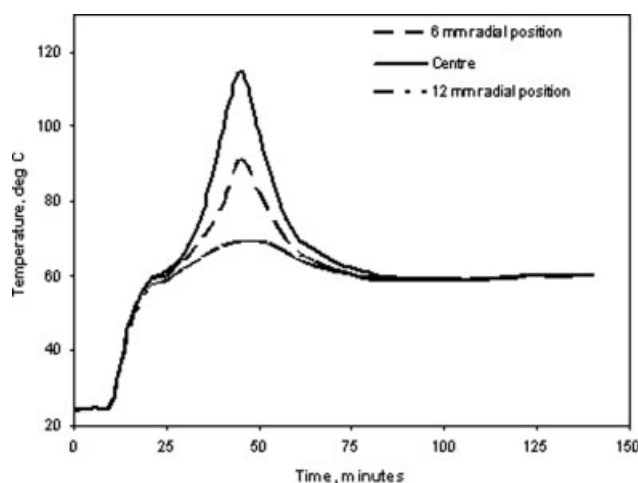


Figure 2 Temperature distribution profiles in the radial direction along the length of the 80 mL monolith for bulk polymerization. Radial points investigated are the center, 6 and 12 mm positions. Figure shows the highest temperature gradient of 55°C established at the center.

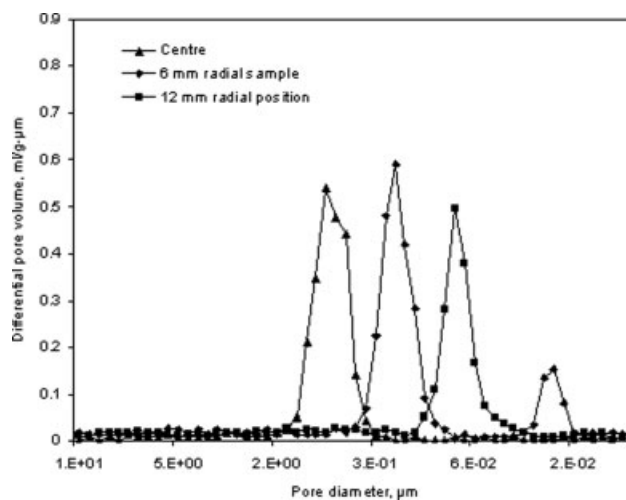


Figure 3 Pore size distribution of samples sliced from the different radial positions (center, 6 and 12 mm) of the 80 mL poly(GMA-co-EDMA) monolith synthesized via bulk polymerization. The different portions of the monolith display different pore size distributions, thereby rendering the entire pore structure nonuniform.

via bulk polymerization were confirmed by preparing 80 mL monolith in the $20 \times 2.5 \text{ cm}^2$ mold using a typical polymerization feedstock with AIBN initiator at an initial temperature of 60°C . An aggressive evolution of exothermic fumes occurred during the polymerization, leading to a monolith with a disfigured surface. The exothermicity of the reaction was enough to increase remarkably the reaction temperature from its initial level and to accelerate the polymerization rate owing to the rapid decomposition of the initiator with an accompanying release of nitrogen gas. The characteristic temperature distribution during the polymerization inside the mold at different radial positions (center, 6 and 12 mm points) is presented in Figure 2. The reactant mixture is prepared at room temperature and placed into a thermostated water bath at 60°C . During the heating process, the temperature of the reactant mixture steadily approaches the water bath temperature. At this point, the initiator becomes thermally unstable and starts to decompose to free radicals that activate polymerization. The high degree of exothermicity associated with the bulk polymerization causes tremendous increase in the polymerization temperature, which accelerates the reaction kinetics and as a result aggravates the evolution of exotherms. This causes a substantial temperature gradient in the radial direction as the polymerization system is no longer able to effectively distribute the heat of polymerization. The self-accelerated reaction retards when the system runs short in monomer concentration and finally the polymerization stops. According to Figure 2, the maximum temperature established at the center was 115°C ; an increase of 55°C over the

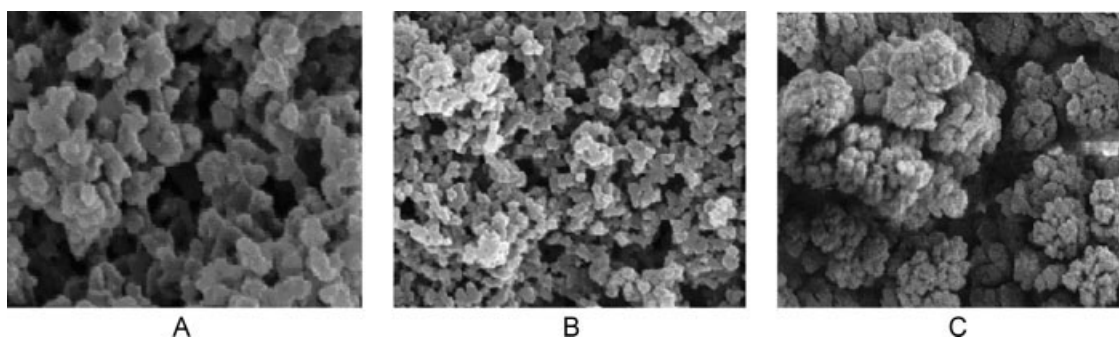


Figure 4 SEM pictures of the 80 mL monolithic polymer synthesized via bulk polymerization. Pictures A, B, and C show the micrographs of samples sliced from the different radial positions; center, 6 and 12 mm, respectively. Pictures display the heterogeneous nature of the pore system.

water bath temperature. The deviation from the desired polymerization temperature is clearly reflected in the radial difference in the pore properties of the monolith. Figure 3 represents the pore size distributions at the different radial positions of the monolith. There is a high degree of inconsistency in the pore size distribution at the different radial positions, as the pore size profiles present different pore size modalities and arrangements in the matrix. Morphological studies of samples sliced from the different radial positions as in Figure 4 displays distinct features of pore interconnectivities for each of the different samples. The monolith thus prepared was practically of no use. The monolith synthesis was carried out at different polymerization temperatures to study the effect of temperature on the extent of exothermicity at a specific radial position. Figure 5 represents the comparison between the tempera-

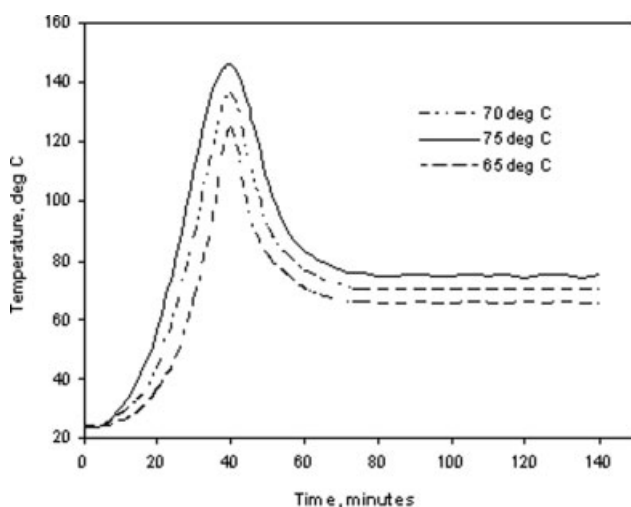


Figure 5 Comparison of experimentally measured temperature distributions at the center of the mold during bulk polymerization of 80 mL monolith at different water bath temperatures; 65, 70, and 75°C. Maximum temperature gradient increases with increasing polymerization temperature.

ture profiles at the center position for polymerizations at 65, 70, and 75°C. A remarkable impact of the polymerization temperature on the reaction rate kinetics and the maximum temperature reached is obvious. Higher polymerization temperature increases the rate of polymerization and the maximum temperature reached. This results in a nonuniform structure of the monolith.

Large-volume methacrylate monolith synthesis via heat expulsion and bulk polymerization

As already elucidated, temperature is an essential factor that controls the pore structure of methacrylate monoliths. Generally, increasing polymerization temperature results in decreasing pore size and *vice versa*.^{16,17} As seen in Bulk Synthesis of Methacrylate Monolith section, during the bulk synthesis of large-volume methacrylate monolith, the occurrence of

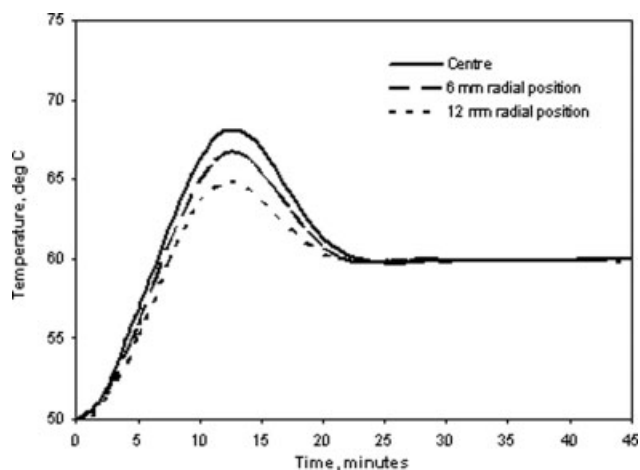


Figure 6 Temperature distribution profiles in the radial direction along the length of the 80 mL monolith synthesized via heat expulsion and bulk polymerization. Radial points investigated are the center, 6 and 12 mm positions. Figure shows the highest temperature gradient of 8.5°C established at the center.

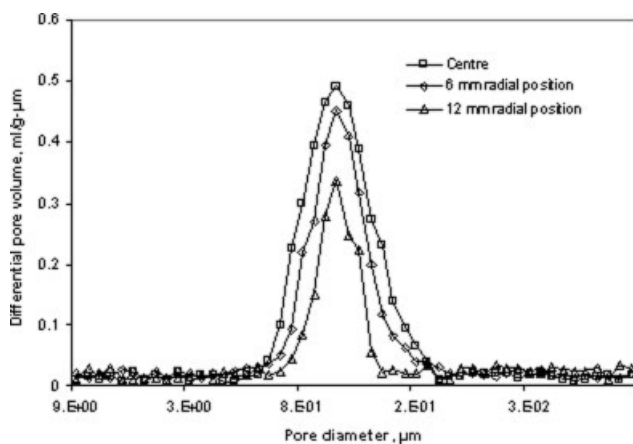


Figure 7 Pore size distribution of samples sliced from the different radial positions (center, 6 and 12 mm) of the 80 mL poly(GMA-co-EDMA) monolith synthesized via heat expulsion and bulk polymerization. The different portions of the monolith display pore size distributions with improved uniformity. An identical modal pore diameter of ~ 400 nm is revealed by the different samples.

high radial temperature gradient is inevitable. Thus, a nonuniform pore structure occurs in the prepared monolith. The large amount of heat generated during the bulk polymerization process can be disintegrated into the heat evolutions resulting from initiator decomposition, monomer–monomer and monomer–initiator interactions within the porogen. To obtain a lower heat generation to cause a lower radial temperature gradient, AIBN/cyclohexanol mixture was preheated separately to initiate AIBN decomposition, resulting heat/fume expelled and the free radical-porogen mixture transferred instantly into the polymerization mold containing preheated monomer mixture at the same temperature as the free radical-porogen mixture. The temperature of the system was increased to the polymerization temperature immediately after the instantaneous bulk addition. In the mold, polymerization

commenced immediately after the free radicals have contacted the monomers. The heat evolved further increases the temperature of the system beyond the polymerization temperature to a maximum less than that observed during the bulk polymerization (Fig. 6). The expulsion of the heat of initiator decomposition considerably reduces the large amount of heat responsible for high temperature gradients during the bulk polymerization. The monolith prepared by this approach is relatively free of deformities, with homogeneity in the pore size distributions of the different samples from the different radial positions as shown in Figure 7. The radial temperature profiles measured during the polymerization confirm that the improvement in pore structure homogeneity indeed results from the decrease in exothermicity. As shown in Figure 6, the maximum recorded temperature is 68.5°C at the center, which is 8.5°C higher than the actual polymerization temperature. Comparing this to that of the bulk polymerization gives 46.5°C reduction in temperature gradient. This radial temperature gradient reduction is undoubtedly attributable to the heat expulsion step included in this methodology. As shown in Figure 8, the SEM pictures of samples from the different radial positions show that the morphology of the different portions is similar. The pores in the matrix are interconnected, forming a porous network of channels.

Large-volume methacrylate monolith synthesis via heat expulsion and gradual addition polymerization

In this technique, AIBN/cyclohexanol mixture was preheated separately to initiate AIBN decomposition and the resulting free radical-porogen mixture pumped continuously and isothermally into the polymerization mold after the heat/fumes from AIBN decomposition has been expelled. The monomer mixture was also pumped simultaneously under

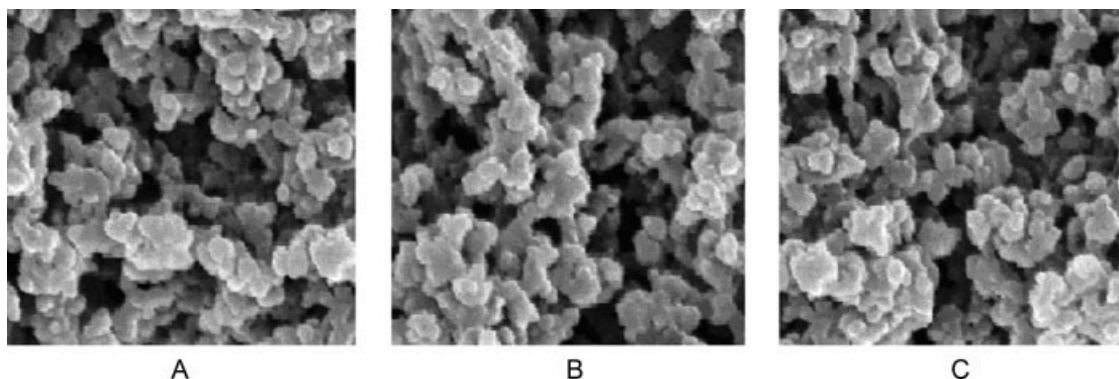


Figure 8 SEM pictures of the 80 mL monolithic polymer synthesized via heat expulsion and bulk polymerization. Pictures A, B, and C show the micrographs of samples sliced from the different radial positions; center, 6 and 12 mm, respectively. Pictures display an improvement in the uniformity of the pore structure.

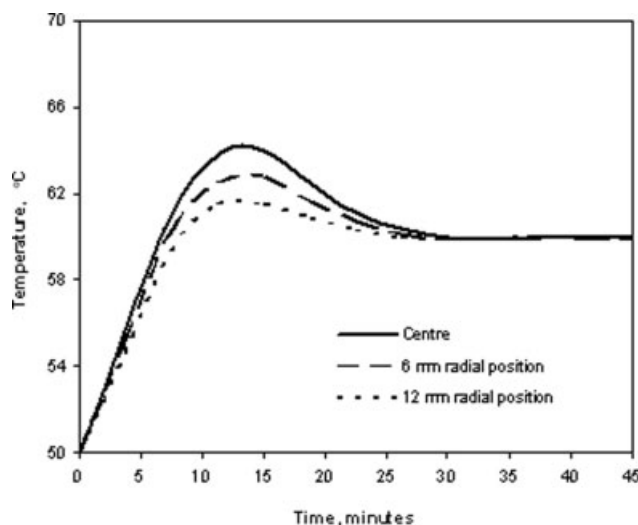


Figure 9 Temperature distribution profiles in the radial direction along the length of the 80 mL monolith synthesized via heat expulsion and gradual addition polymerization. Radial points investigated are the center, 6 and 12 mm positions. Figure shows the highest temperature gradient of only 4.3°C established at the center.

identical conditions into the polymerization mold. In the mold, polymerization commenced immediately after the free radicals have contacted the monomers. The heat evolved further increases the temperature of the system beyond the polymerization temperature to a maximum far less than that observed during the bulk polymerization (as shown in Fig. 9). The expulsion of the heat of decomposition considerably reduces the large amount of heat responsible for high temperature gradients during the polymerization. Also, the continuous and gradual introduction of monomer and free radical/porogen mixtures into the polymerization mold minimizes the heat evolved during the polymerization as the mass of feedstock per unit time is limited. By this technique, the polymer grows slowly upward from the bottom of the mold. The monolith prepared in this manner

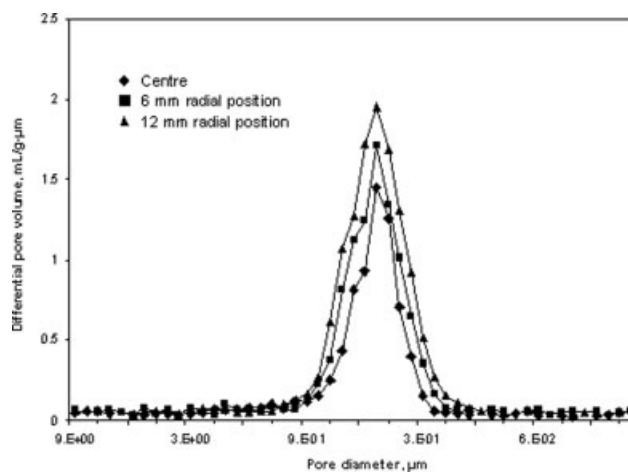


Figure 10 Pore size distribution of samples sliced from the different radial positions (center, 6 and 12 mm) of the 80 mL poly(GMA-co-EDMA) monolith synthesized via heat expulsion and gradual addition polymerization. The different portions of the monolith display identical pore size distribution with extra homogeneity. An identical modal pore diameter of ~ 400 nm is revealed by the different samples.

is free of deformities, with overwhelming homogeneity in the pore size distributions of different samples from the different radial positions as shown in Figure 10. The radial temperature profiles measured during the polymerization confirm that the improvement in pore structure homogeneity indeed results from the decrease in exothermicity. The maximum recorded temperature as shown in Figure 9 is 64.3°C at the center, which is only 4.3°C higher than the actual polymerization temperature. The reduction in the radial temperature gradient and hence the resulting pore structure uniformity is as an improvement over that reported by Peter et al.⁹ for only gradual addition polymerization. This is undoubtedly attributable to the heat expulsion step included in this methodology. SEM pictures (Fig. 11) of samples from the different radial positions show that the

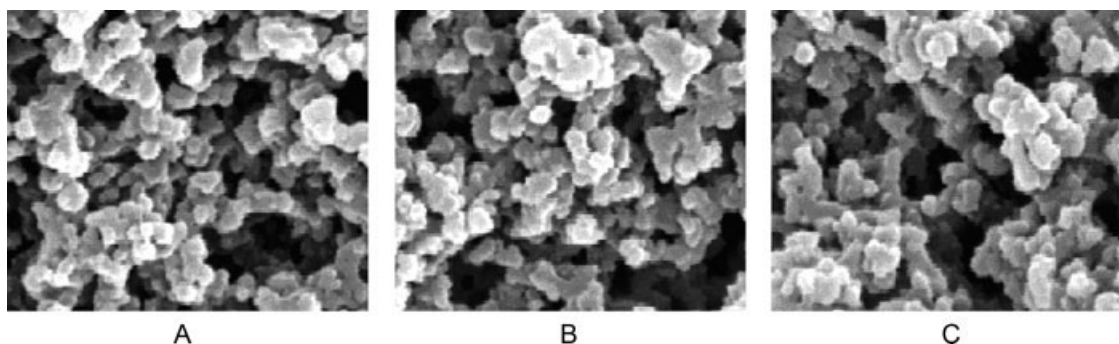


Figure 11 SEM pictures of the 80 mL monolithic polymer synthesized via heat expulsion and gradual addition polymerization. Pictures A, B, and C show the micrographs of samples sliced from the different radial positions; center, 6 and 12 mm, respectively. Pictures display identical pore structure.

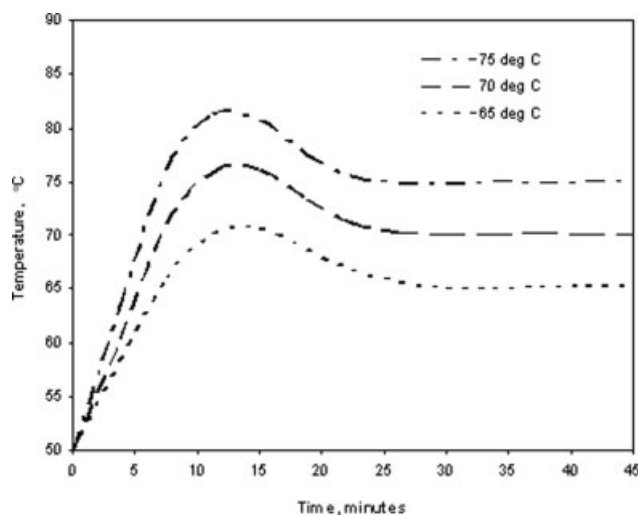


Figure 12 Comparison of experimentally measured temperature distributions at the center of the mold during the 80 mL methacrylate monolith synthesis via heat expulsion and gradual addition polymerization at different water bath temperatures; 65, 70, and 75°C. Increasing the polymerization temperature does not significantly affect the maximum radial temperature gradient.

morphologies of different portions are identical. These results prove that the technique adopted is efficient to establish homogeneity in the pore structure of large-volume methacrylate monoliths. Contrary to our observation in the bulk polymerization process, Figure 12 shows that with the heat expulsion technique, increasing the polymerization temperature does not significantly affect the radial temperature gradient as the greater portion of the heat causing excessive exothermicity is expelled. Maximum radial temperature gradients of only 5.4, 5.9, and 6.7°C were recorded for polymerization temperatures 65, 70, and 75°C, respectively.

CONCLUSIONS

The experimental results show that large-volume methacrylate monoliths with completely homogene-

ous pore structures are produced when the heat of polymerization is effectively controlled. The heat expulsion technique coupled with the gradual addition approach proved exceptionally functional, as it allows the preparation of monoliths of any size that cannot be otherwise obtained. This general technique, combined with the ability to functionalize the monolithic polymers, should allow the production of preparative-scale chromatographic columns. In addition, the slow ascendant growth of the monolith that occurs as a result of the gradual addition provides a platform to produce more advanced mold shape conforming materials.

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