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# Comparison of Conventional and Innovative Technique in Monolith Homogeneity Analysis

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**Abstract**

For more than a decade, monolith up scaling has been a huge hurdle as it is tricky to eradicate the exothermic heat associated with the construction of polymethacrylate monolithic column that builds up instantly and results in cracking of monolith sorbents and heterogeneous pore size distribution. Temperature profiling and average pore size analysis have been the most common methods done to determine the degree of heterogeneity caused by internal heat buildup. In our opinion, the mean pore size alone failed to provide enough information to prove the monolith is indeed non - or homogeneous. In this research, we have incorporated pore size distribution analysis together with temperature profiling and qualitative analysis through SEM (PTS) to make a conclusive judgment on homogeneity of monolith. The findings showed that PTS analysis provided more data and trends that could accurately determine the homogeneity of monolith compared to conventional analysis method.

## Introduction

It has been a well known fact that monolith has much higher ceiling of potential compared to conventional particulate support, especially on scalability. While there were lots of successful applications at smaller scale, it has not been much of a smooth ride for those attempting large scale monolith application (Ongkudon & Michael, 2011; Ongkudon *et al.*, 2013; Burden *et al*., 2012). The main issues stem from the poor heat transfer, a byproduct of large monolith, that results in internal heat buildup which causes several side effect on monolith such as cracking of adsorbent and heterogeneous pore size distribution (Ongkudon *et al*., 2014; Svec & Fréchet, 1995). The main feature of monolith that makes it invincible in terms of chromatographic performance in comparison to particulate support lies in the interconnected pores allowing convective transport to take place (Jungbauer & Hahn, 2007; Mihelič *et al*., 2005; Vlakh and Tennikova, 2008). However, in order to achieve the interconnectivity in large scale, one cannot rely on the conventional bulk polymerization technique which often leads to heterogeneity of pore size distribution. The fact that the interconnected pore channel could facilitate high velocity mobile phase indicates that the pore size must be big enough, hence, larger biomolecules such as protein, DNA, biomass can easily gain access into the monolith which otherwise would not be possible with particulate support (Peter *et al*., 1997; Danquah & Forde, 2008).

A thorough analysis had to be conducted to estimate the magnitude of effect caused by internal heat buildup and external heating on the porous structure. Mean pore size distribution had been the

predominating feature used to assess monolith homogeneity. Mean pore size distribution was rather an accurate representation of the porous structure across the radial section of a small-volume monolith. However, at larger volumes, monoliths comprised between extremely small and large pore sizes, thus, a mean pore size might not reflect the actual porous structure of the monolith. By constructing actual pore size distribution profiles at different locations on the monolith, a clearer overview of the porous structure of large-volume monoliths could be obtained. The results of such analysis could bode well for the development of new solutions of large-volume monolith preparation.

## Methodology

*Chemical*

Ethylene glycol dimethacrylate (EDMA) 98%, glycidyl methacrylate (GMA) 97%, cyclohexanol 99%, azobisisobutyronitrile (AIBN) and methanol AR grade ≥99.5%.

*Monolith synthesis*

The monolith was prepared via free radical co-polymerization of cross-linker EDMA and GMA as functional monomers. EDMA/GMA mixture was combined with an alcohol-based porogen solvent in the proportion of 35/15/50(GMA/EDMA/cyclohexanol) making a solution with a total volume of 160ml. AIBN (1% weight with respect to monomer) was added to initiate the polymerization reaction. The polymer mixture was sonicated for 20 minutes. The mixture of 150ml was gently transferred into 5.0 cm x 10 cm Econo column (BIORAD) sealed at the bottom end. The top end was sealed with a parafilm sheet and placed in a water bath for 3 h at 60$^{o}$C. Same method was repeated for 21/9/70 and 28/12/60 (GMA/EDMA/cyclohexanol) mixture.

*SEM sample preparation*

Polymer resin was extracted and placed in 1.0 L beaker filled with 600ml of methanol followed by placing it inside incubator shaker overnight under 140 rpm and 37$^{o}$C. The next day, methanol was replaced with 600ml of deionised water under same incubation condition for 4 hours. For analysis of monolith morphology, the monolith was oven dried at 70◦C overnight and scanning electron microscopy was done at 15 kV using high resolution scanning electron microscope (Hitachi S-3400N, Japan) according to the manufacturer's instructions.

*Characterization of polymer resin*

Image J software was used to measure the dimension of porous polymethacrylate resin on SEM image captured and saved prior. Prior to measurement, scale has been calibrated in accordance to the magnification power used to view the monolith under SEM.

*Temperature profiling*

Temperature profiles were constructed by measuring the temperature at 9 different partitions of the large scale monolith throughout the polymerization process.

## Result and discussion

*Shortcoming of Mean Pore Size Analysis*

Figure 1 shows the morphology of polymethacrylate resin viewed under SEM from one partition comprising meso- and micropores. Normally, we would have concluded that the monolith sample viewed comprised heterogeneous pore sizes throughout the monolith. It is also rather misleading to interpret and relate this single-spot heterogeneity to temperature build-up effect. As shown in Figure 2, the mean pore size indicated that the pore size varied slightly across the monolith. However, the severity of pore heterogeneity at a single spot of the monolith was rather unknown. This is the major shortcoming of mean pore size analysis. The pore formation of a monolith is affected by many parameters such as temperature, porogen concentration, monomers ratio (EDMA/GMA) etc. Thus it is very important to study the actual pore size distribution at each position on the monolith before a reasonable conclusion can be made regarding the effect of process parameters on the monolith pore structure.

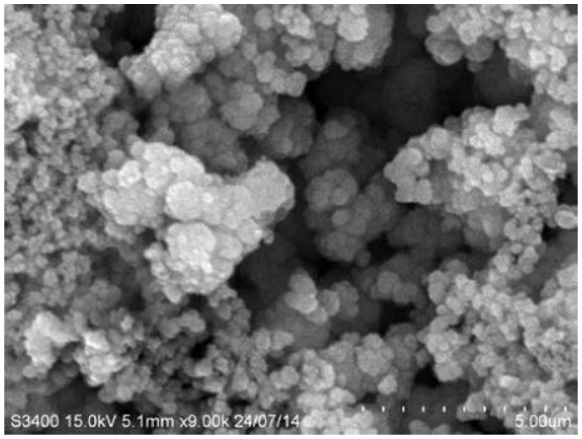


**Figure 1**: The effect of exothermic heat associated with the construction of large scale (150ml) polymethacrylate monolithic column on the surface morphology of methacrylate monolith. Polymerizations were carried out with a constant monomer ratio (EDMA/GMA) of 30/70; porogen concentrations of 70%; polymerization temperature of 60 ◦C; AIBN concentration of 1% (w/w) of monomers. Microscopic analysis was performed at 15 kV.

*PTS analysis*

Figure 2 showed that the top section of 70% porogenic content monolith were non homogeneous due to the differences in average pore sizes among the top outer, top middle and top inner sections. However, when we looked at Figure 3, the overall pore size distribution, the trend across the top section of 70% monolith was similar and consistent. Therefore, it could be concluded that the pore size distribution across the monolith was rather homogenous with a mean pore size of approximately 3 microns. The degree of pore homogeneity could further be improved as temperature build-up was

still noticeable (Figure 4). Lastly, we looked at the SEM images in Figure 5 which were in tandem with results obtained in the above PTS analysis. In conclusion, these observations would not have been possible by one dimensional average pore size analysis.

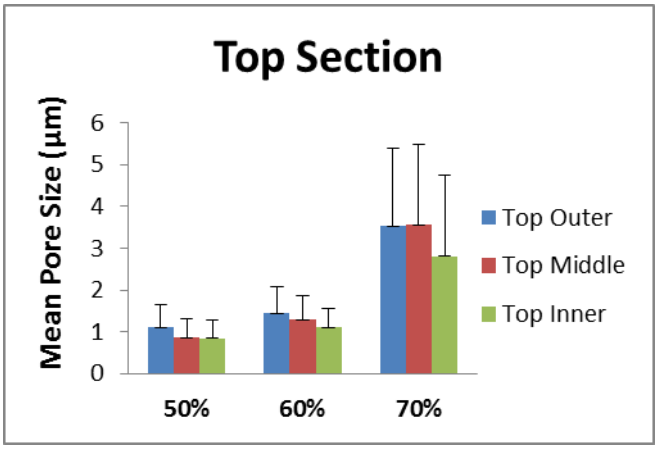


**Figure 2**: The effect of exothermic heat associated with the construction of large scale (150ml) polymethacrylate monolithic column on the surface morphology of methacrylate monolith. Polymerizations were carried out with a constant monomer ratio (EDMA/GMA) of 30/70; porogen concentrations of 70%; polymerization temperature of 60 ◦C; AIBN concentration of 1% (w/w) of monomers. Microscopic analysis was performed at 15 kV.

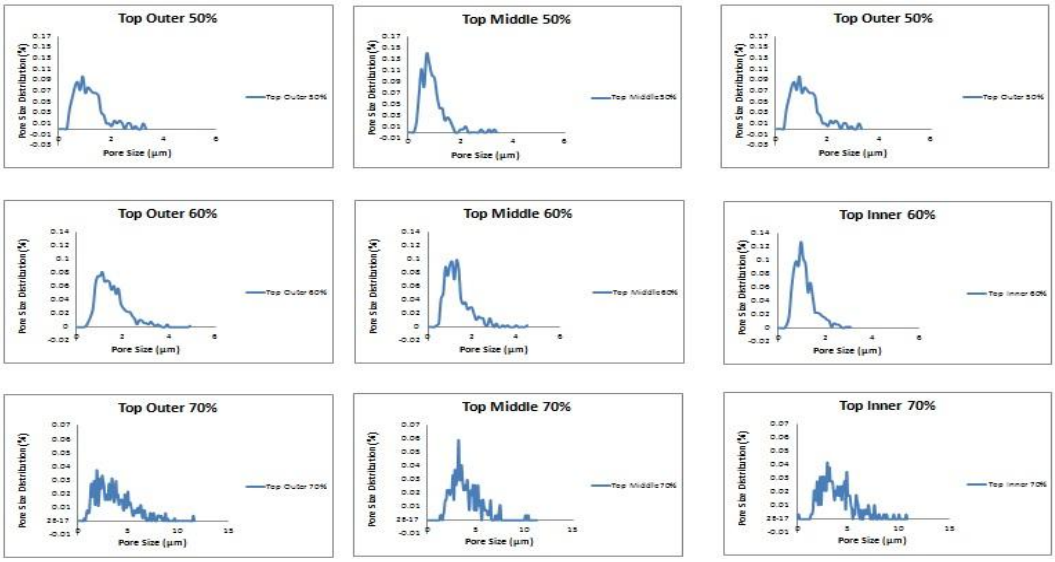


**Figure 3**: The effect of exothermic heat associated with the construction of large scale (150ml) polymethacrylate monolithic column on the pore size distribution of methacrylate monolith (Top Section). Polymerizations were carried out with a constant monomer ratio (EDMA/GMA) of 30/70; porogen concentrations of 50, 60 and 70%; polymerization temperature of 60 ◦C; AIBN concentration of 1% (w/w) of monomers. Microscopic analysis was performed at 15 kV.

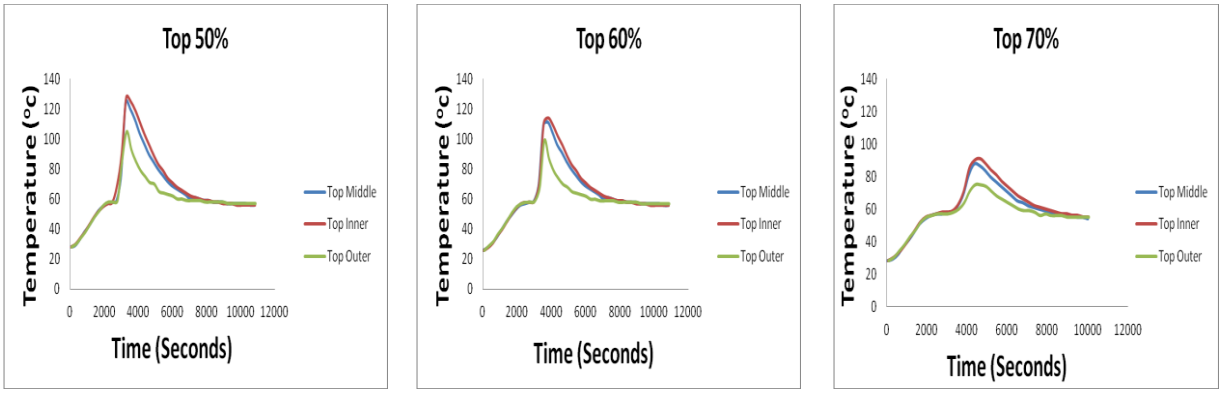


**Figure 4**: The effect of porogenic content (%) on varying degree of heat buildup caused by exothermic heat associated with the construction of large scale (150ml) polymethacrylate monolithic column. Polymerizations were carried out with a constant monomer ratio (EDMA/GMA) of 30/70; porogen concentrations of 50, 60 and 70%; polymerization temperature of 60 ◦C; AIBN concentration of 1% (w/w) of monomers.

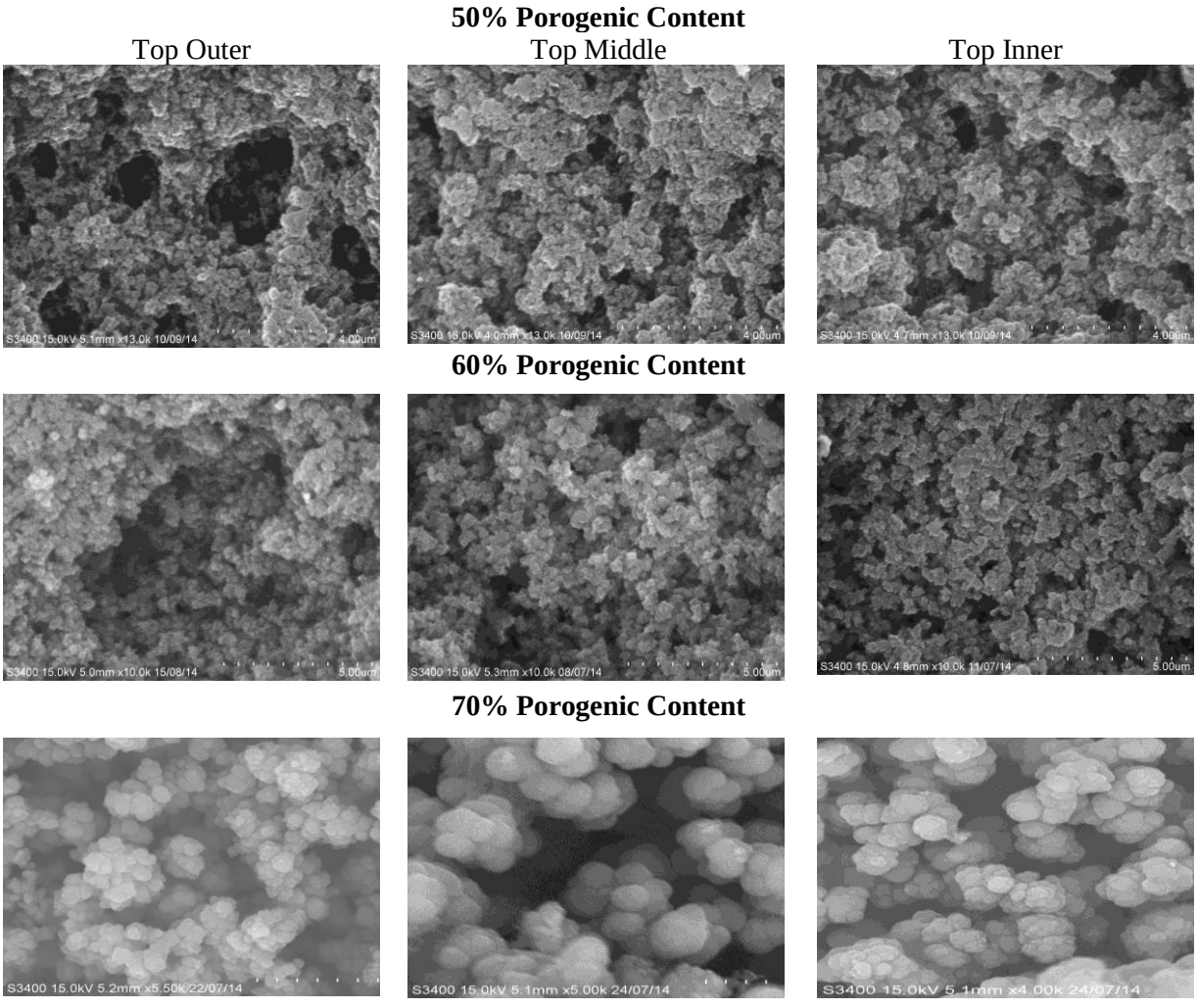


**Figure 5**: The effects of varying porogenic content (50%, 60% and 70%) in the polymerization mixture on the surface morphology of polymethacrylate monolith (top section). Polymerizations were carried out with a constant monomer ratio (EDMA/GMA) of 30/70; porogen concentrations of 50%, 60% and 70%; polymerization temperature of 60 ◦C; AIBN concentration of 1% (w/w) of monomers. Microscopic analysis was performed at 15 kV

## Conclusion

Coherent analytical methodology is vital in understanding the factors that contribute to the morphology and structure of pore throughout the monolith. The PTS analysis proved to be especially important in understanding and correlating process parameters with monolith's pore structure. This would effectively help in designing an appropriate technique for monolith manufacture especially during scale-up.

## Acknowledgements

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