

Preparation of Monodisperse Porous Silica Particles Using Poly(Glycidyl Methacrylate) Microspheres as a Template

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Summary

Monodisperse macroporous poly(glycidyl methacrylate) (PGMA) microspheres were used as a template for preparing porous silica particles. The starting polymer microspheres that were 9.3 μm in size were synthesized by multistep swelling polymerization using a modified Ugelstad technique. Subsequently, silica (SiO_2) was deposited on the surface and inside the PGMA microspheres to produce poly(glycidyl methacrylate)-silica hybrid particles (PGMA- SiO_2). Upon calcination of the PGMA- SiO_2 microspheres, porous silica particles were formed. The morphology, particle size, polydispersity and inner structure of the silica microspheres were investigated by scanning and transmission electron microscopy. Thermogravimetric analysis and dynamic adsorption of nitrogen determined the amount of silica formed and its specific surface area. Compared with the starting PGMA microspheres, the size of the porous silica particles decreased by up to 30 %. These porous silica microspheres are promising for chromatography and biomedical applications.

Key words

Microspheres • Monodisperse • Silica • Poly(glycidyl methacrylate) • Porous

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Introduction

For decades, porous particles, especially those that are spherical in shape, have been of great research interest due to their applicability for separating molecules

(Li *et al.* 2013), catalysis (Lu and To 2009, Bogdan *et al.* 2007), diagnostics (Sinitsyna *et al.* 2012), sensors (Salinas-Castillo *et al.* 2010) and drug delivery systems (Elbert 2011). Depending on the pore size, the particles are classified as macro-, meso- and microporous, for pores > 50 nm, within the range of 2-50 nm and < 2 nm respectively. Moreover, size, particle size distribution, chemical structure and functionality are important features when selecting particles for a required application. For example, functional nanosized particles are often used in medicine (Li *et al.* 2010), while chromatography (Haginaka 2008) requires monodisperse microspheres with a size in the micrometer range adjusted according to the separation purposes.

Many techniques can be employed when preparing polymer microspheres. Suspension, dispersion, precipitation, multistep, emulsion and microfluidic polymerizations are the main methods used to form porous polymer particles. In all cases, the application should be kept in mind prior to choosing the process of production (Gokmen and Du Prez 2012). In addition, the use of different porogens during polymerization leads to the formation of porous beads with the desired pore size and distribution (Dubinsky *et al.* 2009).

Silica particles are typically synthesized using a sol-gel process from alkylorthosilanes. The method was introduced by Stöber and Fink (Stöber *et al.* 1968). The method is primarily used for the preparation of nonporous nanosized silica. In order to produce porous silica beads, an alkylamine porogen is often used in combination with the sol-gel process. Even though literature is full of studies about silica particle preparation (Kota *et al.* 2013, Alotman 2012), it is still quite challenging to synthesize

monodisperse porous silica microspheres in the size range of 3-0 μm . Template techniques are being widely used to control the size and morphology of silica particles. Porous silica can be formed by using different organic templates, such as surfactants (Velikova *et al.* 2013), polymers (Tsiourvas *et al.* 2013) and pre-formed molecules (Shen *et al.* 2015). Monodisperse porous silica microspheres were synthesized using polystyrene cross-linked with divinyl benzene as a template (Meyer *et al.* 2002). Optionally, mesoporous silica spheres were obtained in the presence of highly interconnected macroporous polyacrylamide (Ahmed *et al.* 2010).

Silica is biocompatible, optically transparent and relatively environmentally inert. Last but not least, silica is chemically reactive and amenable to the introduction of various functional groups required by biological applications (Knopp *et al.* 2009). Another advantage of porous silica particles is that iron oxide can be prospectively precipitated inside the pores to provide magnetic properties. The easy functionalization and magnetic properties of microparticles can be then exploited in the separation of cells or proteins and for diagnostics. Recently, we have used functional magnetic porous microspheres to capture cancer cells (Horák *et al.* 2013, Kuan *et al.* 2014), biomarkers for detecting Alzheimer's disease (Horák *et al.* 2014, de la Escosura-Muñiz *et al.* 2015) and as affinity sorbents (Horák *et al.* 2012, Kučerová *et al.* 2014).

With the attempt to design new systems that can be used for the above-mentioned purposes, this paper reports on the preparation of monodisperse porous silica particles by using poly(glycidyl methacrylate) microspheres as a template. The morphology, particle size, polydispersity and inner structure of the silica microspheres were investigated by scanning and transmission electron microscopy. The porous properties were determined by measuring the specific surface area of the silica particles.

Methods

Preparation of porous silica microspheres

Tetramethyl orthosilicate (TMOS) and trimethoxy(methyl) silane (TMMS) were supplied by Sigma-Aldrich (St. Louis, USA). Starting PGMA microspheres were synthesized by a multistep swelling polymerization that was first reported by Ugelstad (Ugelstad *et al.* 1988) with some modifications (Grama *et al.* 2014b). The silica was formed on the surface and

in the inner pores of the starting PGMA microspheres by a three-step procedure based on the Stöber technique (Stöber *et al.* 1968). The polymer-silica hybrid particles were obtained by filling the PGMA pores with a hydrolysate preformed from TMOS or TMMS under mild conditions, that is, in highly dilute HCl at 60 °C. It is necessary to mention that no porogens or surfactants were used during the silica formation. The synthesis of monodisperse macroporous poly(glycidyl methacrylate) (PGMA) and poly(glycidyl methacrylate)-silica (PGMA-SiO₂) microspheres was described elsewhere (Grama *et al.* 2014b) and (Grama *et al.* 2014a). The dried PGMA-SiO₂ microspheres were calcinated at 650 °C for 6 h at a heating rate of 10 °C/min under air. After calcination, the remaining product consisted of porous silica microspheres that were free from the PGMA polymer microspheres.

Characterization of microspheres

The microspheres were viewed under an Opton III light microscope (Oberkochen, Germany), a 200S Quanta scanning electron microscope (SEM; FEI; Brno, Czech Republic) equipped with an energy dispersive X-ray analysis (EDAX) and a Tecnai Spirit G2 transmission electron microscope (TEM; FEI). The particle size distribution was determined by the Atlas software (Tescan; Brno, Czech Republic) and at least 500 particles were counted in each experiment. The number-average diameter (D_n), weight-average diameter (D_w) and polydispersity index (PDI) were calculated as $D_n = \sum D_i/N$, $D_w = \sum D_i^4/\sum D_i^3$, $PDI = D_w/D_n$, where D_i is the diameter of i -th particle and N is the total number of particles counted. A thermogravimetric analysis (TGA) was performed in air using a Perkin Elmer 7 thermogravimetric analyzer ranging from 30 to 850 °C at a heating rate of 10 °C min⁻¹. The specific surface area (S_{BET}) of the microspheres was determined by liquid nitrogen adsorption (77 K) using a Gemini VII 2390 Analyser (Micromeritics; Norcross, GA, USA).

Results

The obtained PGMA microspheres were 9.3 μm in diameter with a low polydispersity index (PDI = 1.004) (Fig. 1a, Table 1) conforming to their monodispersity. An amplified view revealed a rather rough surface of the particles with distinct pores (Fig. 1b). A TEM micrograph from a microsphere cross-

section showed its inner structure consisting of large pores (Fig. 1c). The porous structure of PGMA

microspheres was confirmed by measurements showing the specific surface area to reach 85.6 m²/g (Table 1).

Table 1. Properties of microspheres.

Microspheres	Silanization agent	D_n^a (μm)	PDI ^b	SiO ₂ (wt.%)		S_{BET}^c (m ² /g)
				EDAX ^d	EDAX ^e	
PGMA	-	9.3	1.004	-	-	85.6
PGMA-SiO ₂	TMOS	8.6	1.004	35.8	13.7	4.5
SiO ₂	TMOS	7.9	1.005	19.1	26.0	331.4
PGMA-Me-SiO ₂	TMMS	9.7	1.005	14.4	18.4	18.2
Me-SiO ₂	TMMS	6.0	1.006	18.9	26.3	323.6

^a Number-average diameter, ^b Polydispersity index, ^c Specific surface area, ^d Energy dispersive X-ray analysis coupled with SEM, ^e Energy dispersive X-ray analysis coupled with TEM.

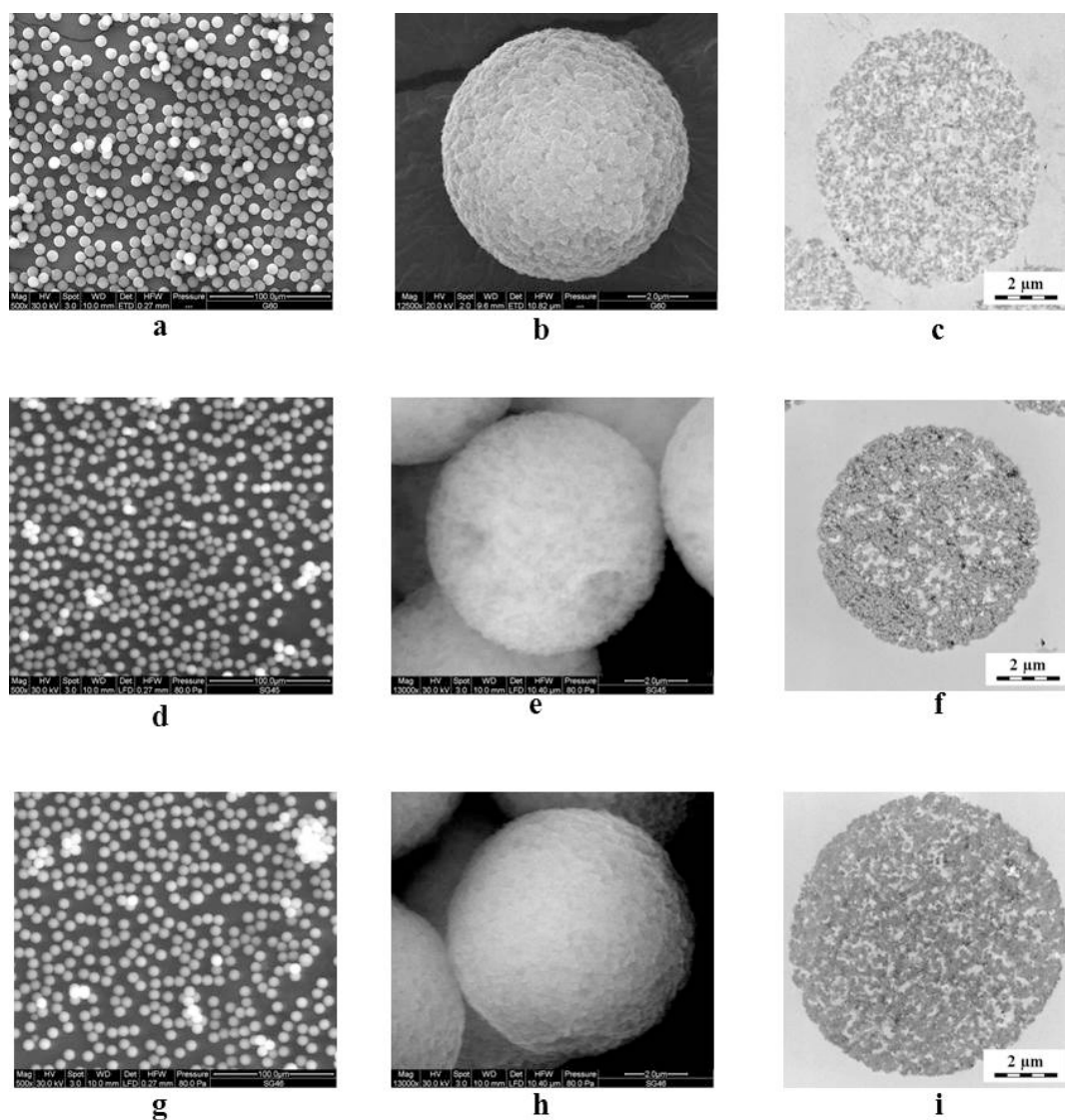


Fig. 1. SEM (a, b, d, e, g, h) and TEM micrographs (c, f, i) of starting PGMA (a-c), PGMA-SiO₂ (d-f) and PGMA-Me-SiO₂ (g-i) microspheres; (c, f, i) are particle cross-sections.

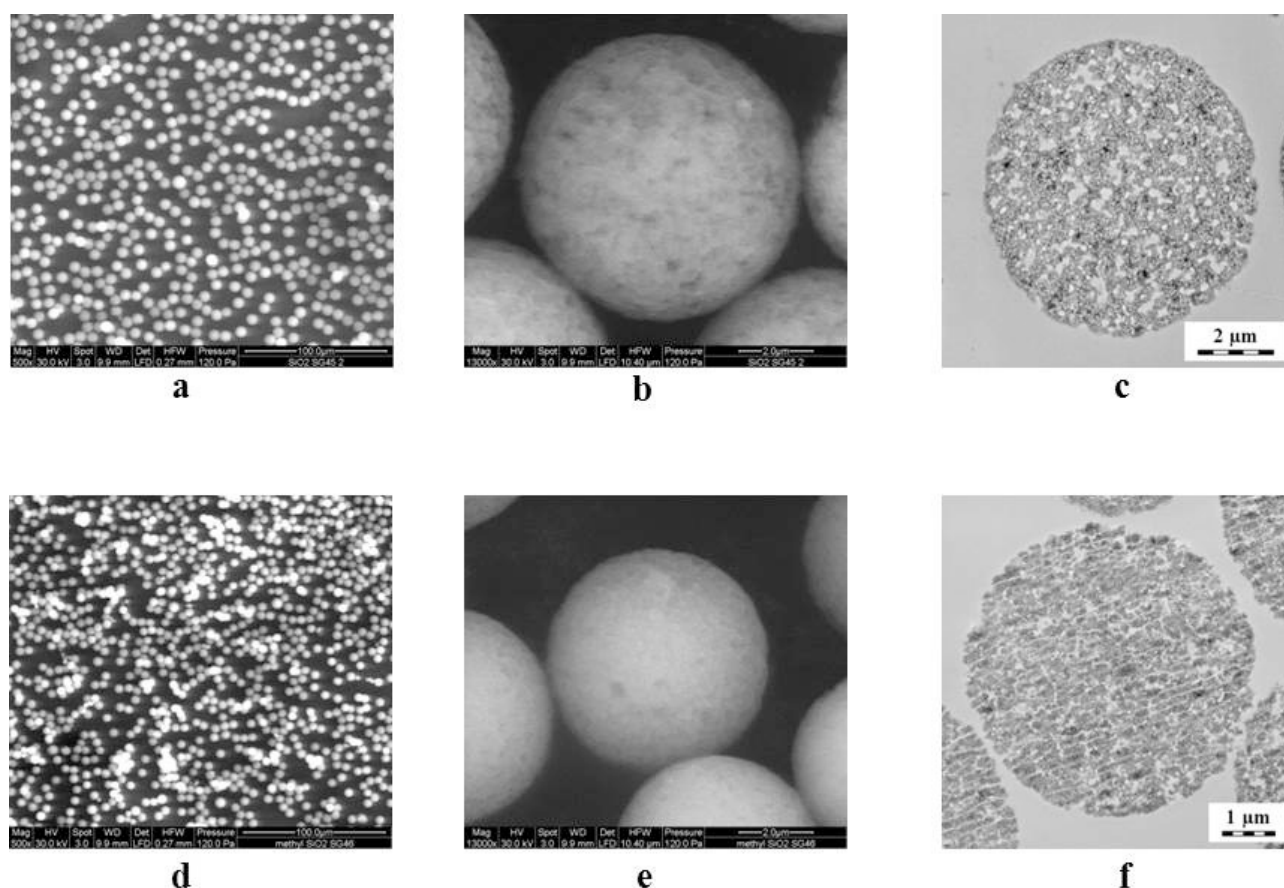


Fig. 2. SEM (**a, b, d, e**) and TEM (**c, f**) of SiO₂ (**a-c**) and Me-SiO₂ microspheres; (**c, f**) are particle cross-sections.

The resulting PGMA-SiO₂ and poly(glycidyl methacrylate)-methyl-silica (PGMA-Me-SiO₂) microspheres are shown in Figures 1d-f and 1g-i, respectively, documenting that silica was formed both on surface and inside the PGMA pores. The size of the polymer-silica hybrid microspheres was 8.6 μm for PGMA-SiO₂ and 9.7 μm for PGMA-Me-SiO₂ microspheres, while the monodispersity remained unchanged (Table 1, Figs 1d and 1g). SEM and TEM cross-section micrographs revealed that PGMA pores disappeared by being filled with silica (Figs 1e, 1f, 1h and 1i). The pore properties of the hybrid microspheres were investigated by nitrogen adsorption. The specific surface areas were 4.5 and 18.2 m²/g for PGMA-SiO₂ and PGMA-Me-SiO₂ particles, respectively (Table 1).

Upon calcination of PGMA-SiO₂ and PGMA-Me-SiO₂ microspheres, porous SiO₂ and Me-SiO₂ particles were obtained. During removal of the polymer template, particles partially shrank, reaching a diameter of 7.9 μm for SiO₂ and 6.0 μm for Me-SiO₂ spheres, while PDI remained unchanged (Table 1, Figs 2a and 2d). While the shrinking of SiO₂ particles was not pronounced (700 nm), the size of the Me-SiO₂ particles was smaller

by 30%. The porous character of the particles was confirmed both by electron microscopy and a surface area analysis. After removing the PGMA template, SEM and TEM cross-section micrographs revealed that the SiO₂ particles contained rather large and irregular pores, while the Me-SiO₂ microspheres had smaller pores (Figs 2c and 2f). Porous silica formation was additionally confirmed by the relatively high specific surface area, 331.4 m²/g for SiO₂ and 323.6 m²/g for Me-SiO₂ microspheres (Table 1).

SEM and TEM coupled with EDAX provided information about the content of silicon in the PGMA-SiO₂ and SiO₂ microspheres. It amounted to 16.7 (SEM) and 6.4 wt.% (TEM) in PGMA-SiO₂, which represented 35.8 and 13.7 wt.% of SiO₂. The content of Si in SiO₂ particles (8.9 and 12.2 wt.% according to SEM and TEM, respectively) corresponded to 19.1 and 26.0 wt.% amount of SiO₂. In PGMA-Me-SiO₂ and Me-SiO₂ particles, the amount of silica increased from 14.4 (SEM) and 18.4 wt.% (TEM) for PGMA-Me-SiO₂ to 18.9 and 26.3 wt.%, respectively, for Me-SiO₂ microspheres (Table 1).

TGA was used to study the silica content in the PGMA-SiO₂ microspheres (Fig. 3). At temperatures below 230 °C, a rather small weight loss was found that

was attributed to the evaporation of solvents. Starting from 250 °C, fast decomposition of organic compounds was observed. Full decomposition occurred at 550 °C; however, a residual amount of SiO₂ still persisted at 750 °C. The amount of silica remaining at the end of the PGMA decomposition was 33.4 wt.% for SiO₂ and 40.5 wt.% for Me-SiO₂.

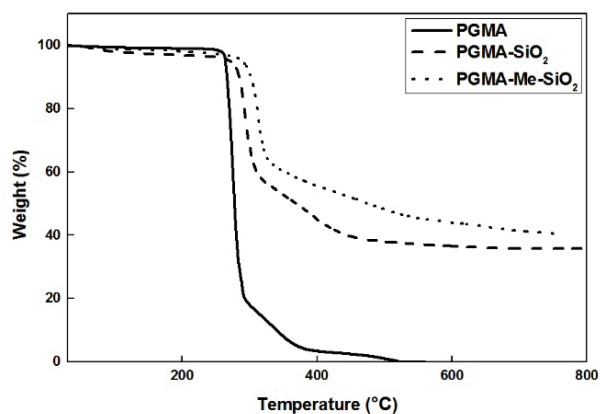


Fig. 3. TGA of PGMA, PGMA-SiO₂ and PGMA-Me-SiO₂ microspheres.

Discussion

PGMA microspheres are of growing interest as a matrix for producing inorganic particles due to their macroporosity and monodispersity. In this report, 9 μm PGMA microspheres were successfully used as templates for developing porous silica particles in the absence of any auxiliary surfactant or porogen. The macroporosity of the PGMA microspheres plays an important role in the formation of porous silica particles.

During the silanization process, when PGMA-SiO₂ and PGMA-Me-SiO₂ hybrid microspheres are formed, the monodispersity remains unchanged, thereby confirming the absence of silica outside of the PGMA particle surface. It is interesting to note that while the diameter of PGMA-SiO₂ microspheres decreased ($D_n = 8.6 \mu\text{m}$), the size of PGMA-Me-SiO₂ increased ($D_n = 9.7 \mu\text{m}$). This can be explained by different mechanisms of condensing silanol into silica. While silica was formed within the microsphere bulk filled with TMOS, the silica from TMMS had a tendency to phase-separate and coat the particles, thus increasing their size (Grama *et al.* 2014a). This behavior influenced the final size of the silica particles. The lower specific surface area of PGMA-SiO₂ and PGMA-Me-SiO₂ compared with the

starting PGMA microspheres were due to the formation of silica on the microsphere surface, which was in agreement with the SEM micrographs. Incorporating silica in PGMA particles increased their thermal stability. This increase of thermal stability of the hybrid microspheres was documented by a shift of decomposition temperature in PGMA particles to higher values.

After the calcination of the PGMA matrix, porous silica microspheres were obtained. During removal of the polymer template, the particles partially shrank. The difference between the final size of the SiO₂ and Me-SiO₂ particles can be attributed to the fact that while TMOS formed silica mostly inside the PGMA pores, TMMS had the tendency to coat the particle surface, as previously mentioned. If monodisperse porous silica microspheres were prepared from tetraethylorthosilane using ethylenediamine-functionalized PGMA microspheres as a template, calcination of the polymer produced silica with a porous or hollow structure maintaining the same size as the initial polymer microspheres (He *et al.* 2012). In order to avoid the shrinking, tetra-*n*-butylammonium bromide or tetramethylammonium hydroxide were present during silanization. In our approach, TMOS and TMMS were silanization agents used to control the morphology of the silica particles. The SiO₂ produced from TMOS had a tendency to fill in the pores of the PGMA microspheres, while Me-SiO₂ mostly formed on surface of the PGMA particles. This is the reason why SiO₂ particles, after removal of the polymeric template, maintained the same size as the initial microspheres, while the diameter of the Me-SiO₂ particles dramatically decreased. The pore structure of the silica particles depended on the silanization agent that was used. According to the specific surface area and the TEM of particle cross-sections, larger irregular pores were present in the silica formed from the TMOS silanization agent, in contrast to the small pores of the Me-SiO₂ microsphere. The Si signal detected on SiO₂ and Me-SiO₂ microspheres by EDAX-coupled SEM and TEM was strong. Only in SiO₂ particles did the content of silicon decrease compared with PGMA-SiO₂.

In conclusion, porous silica particles were developed by using monodisperse, macroporous PGMA microspheres as a template. The porous silica particles maintained their monodispersity after removal of the PGMA microspheres, and in fact they even shrank in size by 30%. The selection of the silanization agents influenced the size and porous structure of the final silica microspheres. The amount of formed silica determined

by electron microscopy coupled with EDAX and a thermogravimetric analysis reached 40 wt.%. The high values of the specific surface area of SiO₂ and Me-SiO₂ microparticles suggest the formation of a porous structure. The newly-developed porous silica particles are intended for applications in chromatography, biology and as a catalyst. The potential range of applications of silica microspheres can be further extended either by functionalization and/or precipitation of magnetic iron

oxides inside the pores of the microspheres.

Conflict of Interest

There is no conflict of interest.

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