Porous Crosslinked Spherical Resins for Diversified Applications: Packing Materials for Size Exclusion Chromatography

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Summary: Improvements in the synthesis of porous polymers for different applications have been carried out in our laboratory. Beads of poly(styrene-co-divinylbenzene) with morphology adequate to the application at hand were prepared. Packing materials for size exclusion chromatography (SEC) were prepared by single-step swelling and polymerization (SSWP) and by modified suspension polymerization (MSP). High values of exclusion limit (10^6 and $8.0\text{x}10^6$) were attained for SEC columns packed with poly(styrene-co-divinylbenzene) synthesized using high proportions of polystyrene, as porogen agent, and divinylbenzene. The maximum values of exclusion limits were attained for SEC columns packed with beads prepared by SSWP method.

Introduction

The growing interest for porous polymer beads in the last three decades is due to their great number of diversified applications as packing materials for size exclusion chromatography (SEC), as supports for solid-phase organic synthesis, polymer-supported catalysts, polymer-immobilized extractants, adsorbents, ion exchange and chelating resins, etc. These polymers are produced by free radical aqueous suspension copolymerization of an adequate monovinyl monomer with a divinyl monomer or by chemical modification of a monovinyl-divinyl copolymer.[1-6]

A great number of ion-exchangers and polymer reagents are based on styrene-divinylbenzene copolymers (supports) which characteristics depend on the support morphology and on the morphological changes of its three-dimensional network after introducing the active sites. The specified application of these copolymers are closely related to their morphology and swelling properties.[7]

The morphology of these copolymers is governed by the occurrence of phase separation during the copolymerization reaction. That process is achieved by combining high

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proportion of crosslinking monomer and porogens in the polymerization mixture. Typical porogens employed in suspension polymerization can be classified into three types: solvating diluents (thermodynamically good solvents); non-solvating diluents (thermodynamically poor solvents); soluble polymers or mixtures thereof. At the end of the polymerization process the porogen agent is removed from the polymer network and the porous structure is attained. The type and amount of porogen and the proportion of crosslinking monomer relative to the vinyl monomer are the most important reaction variables in determining the type of morphology produced, that may vary from gel-type to macroporous with many degrees between them.[4] The gel-type resins do not have pores in dry state, they must be swollen in a good solvent in order to present porous properties.[8]

In the macroporous polymers, the macropores are often accessible to both solvents and non-solvents and in general the whole structure is not susceptible to the dramatic changes observed with gel-type resins when the nature of the surrounding medium is changed. However, the choice of using a gel-type or a macroporous polymer depends very much on the final application. In a swelling solvent (good solvent) gel-type beads can often be loaded or chemical modified to higher levels than macroporous ones. Nevertheless, the latter are generally much less sensitive to the choice of solvent to be used in the process at hand. Swollen gel-type resins are less affected to sudden shock but cannot be subjected to steady and high pressures. In opposition, the rigid macroporous beads are brittle and fracture under sudden stress, but can withstand considerable steady pressures.[8]

The objective of this paper is to describe the synthesis of porous beads with small particle size and low size distribution with varied morphologies adequate as packing material for SEC columns.

General procedures for the synthesis of porous polymer beads

Polymer beads are synthesized by free radical initiators through a suspension polymerization process. That process produces spherical particles with sizes dependent on the polymerization conditions. Styrene and acrylic monomers and divinylbenzene, as crosslinking agent, are among the most employed monomers because of the variety of reactions which can be carried out to introduce the functional groups (active sites) on the polymer chain.[9]

The monomers solution (organic phase), also containing a crosslinking agent, a porogen agent and an initiator is added under stirring to a reactor, fitted with a mechanical stirrer, N_2 inlet and a condenser, already containing the aqueous phase (AP), consisting of suspension stabilizers (to avoid bead agglomeration) and a soluble salt (to diminish the monomers solubility in water, by salting out effect), to suspend the organic phase (OP) which, under stirring, forms spherical droplets where the polymerization will occur. The suspension, under stirring, is then heated to promote the polymerization. After polymerization completion, the beads are separated, by filtration, washed to remove stabilizers, porogen agents and unreacted monomers, sieved and finally dried. The bead size is controlled mainly by stirring speed, AP/OP ratio and suspension stabilizers. High stirring speeds and high AP/OP ratios generally produce a smaller bead size. The crosslinking degree of the polymer beads is controlled by varying the proportion of crosslinker in the monomer solution. The beads porosity is determined mainly by the amount and type of porogen system and by the proportion of crosslinker. The porogens can be solvating diluents, non-solvating diluents, soluble polymers or mixtures thereof. The produced beads will not present pores in dry state if the proportion of crosslinker is low and the porogen is a good solvating agent for the polymer chains, in this case the produced beads are gel-type, with no pore structure. At high proportion of crosslinker porous beads can be produced if the porogen is adequately chosen. The pore size varies from less than 20 Å (micropores) to more than 500 Å (macropores). Solvating diluents can produce beads with low pore volume (up to about 0.8 mL/g) and high surface areas (50 – 500m^2/g), non-solvating diluents tend to produce beads with high pore volumes (0.6 – 2.0 mL/g) and surface areas varying from 10 to 100 m^2/g and soluble polymers can result in beads with a pore volume up to 0.5 mL/g, low surface areas (0 – 10m^2/g) and large average pore diameter.[9,10] Table 1 shows the relation between pore size and porosity.

Table 1: Relation of porosity to pore size

Gel porosity	**Pore diameter (Å)**		
	Macroporosity	**Mesoporosity**	**Microporosity**
No pores in dry state	D > 500	20 < D < 500	D < 20
	Macropores	Mesopores	Micropores

Mechanism of pore structure formation

The mechanism of pore formation was described by Kun and Kunin [11] as a process in three steps. During the early stages of the polymerization, a polymer composed of linear chains with pendant vinyl groups is formed. As the polymerization progresses intramolecularly crosslinked microgels and higher molecular weight linear chains soluble in the monomer/diluent mixture are produced. The phase separation occurs and two phases are formed in the polymerizing droplets: a copolymer-rich phase and a diluent phase with a very low polymer concentration, the monomers being distributed between the two phases. Since solvated and very lightly reticulated polymers can behave as a liquid the interfacial tension on polymer-rich phase gives it the low-energy spherical form, and the polymer separates as a mass of spherical microgels (microspheres). At a determined value of conversion of monomer to polymer macrogelation occurs and are produced gel-type beads formed by the agglomeration of microgels (microspheres), this is the first step of the pore structure formation. In the second step, the microspheres are bound together through the polymerization of the monomer molecules solvating the polymer chains. It is during this stage that the porous structure is actually formed.[11] The third step involves the polymerization of residual monomer that is completed by increasing the polymerization temperature. The mechanism of pore structure formation is summarized in Figure 1.

1st STAGE

♣microgel formation
♣phase separation (sperical microgels)
♣microgels agglomeration (macrogelation)
microspheres

2nd STAGE

♣microspheres are bound together
(solvating monomer polymerization)
pore structure

3rd STAGE

♣residual monomer polymerization
♣elimination of porogen agent
macroporous polymer beads

Figure 1: mechanism of pore structure formation

Morphological features of porous polymer beads

Porous polymer beads are constituted of agglomerates (clusters) of microgel spherical particles glued together at their interfaces. Figure 2 presents electron micrographs of two porous poly(styrene-co-divinylbenzene) beads with different porosities.[12] These micrographs reveal the clustered structure and the difference in the size of the agglomerates and pores of the two beads. The visual appearance of those polymer beads is determined by the size of these clusters, and the beads pores are the voids within and between the clusters and the holes between the microspheres from which the agglomerates are formed. Thus, as it was already mentioned in the mechanism of pore structure formation, the porous beads are a two phase material – the gel phase (clusters of microspheres) and the pore phase.[7]

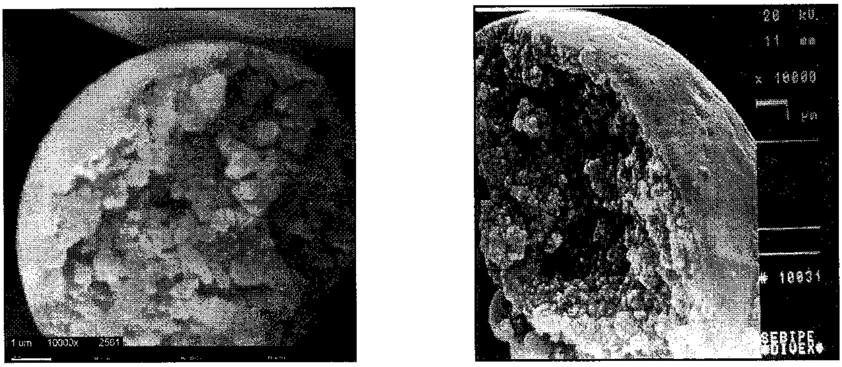
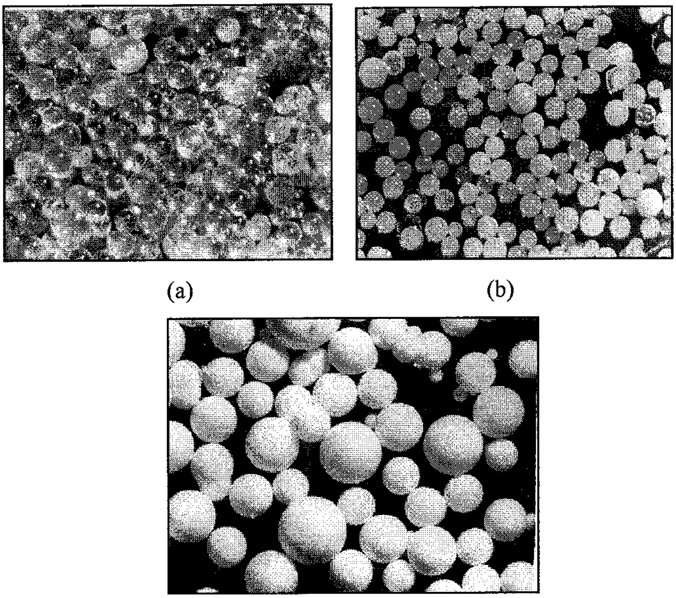


Figure 2: Electron micrographs of two different porous poly(styrene-co-divinylbenzene) beads[12]

When the diameter of the beads internal clusters is approximately 2000 Å or larger, the beads are opaque, when that diameter is smaller than 2000 Å, the beads are translucent. The gel-type beads with no internal pore structure (no pores in dry state) appear transparent (Figure 3).[7]



(a) (b)

(c)

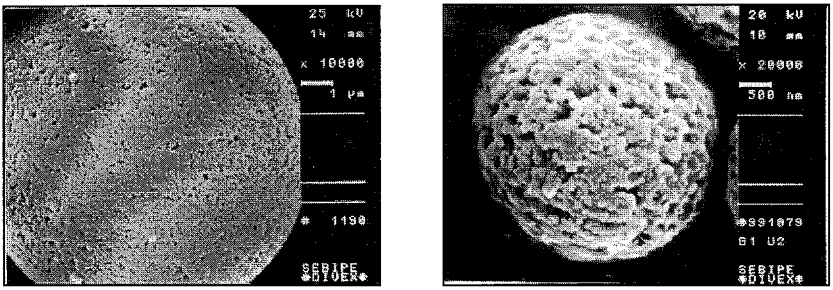
Figure 3: Optical micrographs of beads with different morphologies: (a) transparent gel-type beads, (b) translucent porous beads, (c) opaque porous beads

Styrene-divinylbenzene copolymers as packing materials for SEC

In general, porous polymers are characterized by a relatively broad pore size distribution. All pores in such materials are typically interconnected and the access to the different pores is controlled by the hydrodynamic volume of the dissolved molecules. The molecules will only penetrate those pores that are able to accommodate their size, while smaller pores remain inaccessible for steric reasons. The principle of size exclusion has been adopted in numerous processes from sieving to advanced membrane technologies. This concept is the basis of the size exclusion chromatography (SEC).[6]

The first report on gel filtration, now denominated size exclusion chromatography, was published in 1959.[13] Further improvement in SEC happened in 1964 with the introduction of macroporous beads of poly(styrene-co-divinylbenzne) by Moore.[14] Those beads were synthesized by aqueous suspension polymerization, a process that produces polymer particles with diameters in the range of 50 to 500 µm and a broad particle size distribution. However, to carry out high performance SEC small copolymer beads with sizes in the range of 5 to 10 µm and with narrow size distribution are required.

Our group (IMA/UFRJ) synthesized styrene-divinylbenzene copolymer beads with sizes in the range from 5 to 10 μm by two different methods: modified suspension polymerization (MSP) and single-step swelling and polymerization (SSWP) (Figure 4).



(a) (b)

Figure 4 : Electron micrographs of poly(styrene-co-divinylbenzene) beads produced by (a) MSP and (b) SSWP [12]

The MSP method, developed by Tanaka and Takeda[15] is a modification of conventional suspension polymerization (CSP). In that method the polymerization is preceded by a high speed stirring (20,500 rpm) dispersion of the organic phase in the aqueous phase at low temperature, to avoid premature polymerization, for a specified time with the objective of obtaining small droplets with sizes around 10 μm. Afterwards, these droplets are transformed in polymer beads, with sizes in that same range, as the polymerization takes place as a CSP.[16,17]

The SSWP method is a two stage technique.[18] In the first stage polystyrene seeds with narrow size distribution (Figure 5) are synthesized by dispersion polymerization in an organic medium. The produced seeds with sizes in the range of 1 to 2 μ are swollen with an emulsified mixture of monomers (styrene and divinylbenzene), initiator and diluent and then submitted to the suspension polymerization conditions to produce beads in the desired size range and with narrow size distribution. An schematic representation of the porous beads synthesis by SSWP method is shown in Figure 6.[12,18]

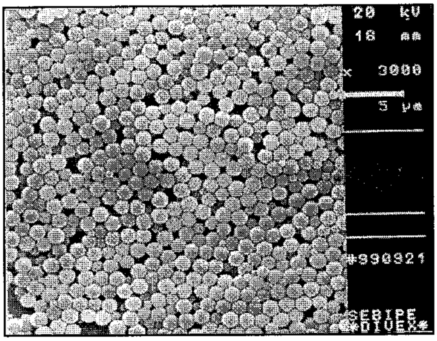


Figure 5: Electron micrograph of polystyrene seeds produced by dispersion polymerization [12]

Poly(styrene-co-divinylbenzene) beads synthesized by SSWP method

The first stage of the SSWP method to produce poly(styrene-co-divinylbenzene) beads is the synthesis of polystyrene seeds by dispersion polymerization initiated by azobisisobutyronitrile (AIBN) in ethanol stabilized by polyvinylpyrrolidone (PVP) of high molecular weight.[19,20] Table 2 shows three conditions employed to prepare polystyrene seeds and their respective characteristics. Figure 6 shows the schematic representation of the SSWP method to produce poly(styrene-co-divinybenzene) beads with small sizes.

Table 2: Polymerization conditions and characteristics of polystyrene seeds[a] [12]

Seeds code	Styrene[b] % V/V	Styrene mL	Ethanol mL	PVP[c] g	Seed diameter µm	Span	M_n x 10^{-4}	M_w x 10^{-5}
PS-34	10	15	135	9	0.94	0.54	6.6	3.44
PS-42	20	60	240	18	1.10	0.62	2.4	0.98
PS-10[d]	20	30	120	1.8	1.73	1.40	2.2	1.46

(a)Initiator = AIBN; [AIBN] = 0.63 %molar (relative to styrene); polymerization temperature = 70°C; stirring speed = 50rpm; polymerization time = 24 h. (b)%Volume relative to (styrene + ethanol). (c) PVP = polyvinylpyrrolidone; M_w = 1.3×10^6; concentration = 60g/L (styrene + ethanol). [d]PVP (K-30), M_w=4.0×10^4, concentration = 60g/L styrene

In the second stage the polystyrene seeds are swollen with an emulsion of the organic phase solution (styrene, divinylbenzene, initiator [2,2'-azobis(2,4-dimethylvaleronitrile) – ADVN], toluene, isoamyl alcohol as compatibilizer and poly(vinyl alcohol)) in water containing poly(vinyl alcohol) (stabilizer) and sodium lauryl sulphate (emulsifier). The polystyrene seeds play two different roles in SSWP method, as templates for the copolymer beads and as porogen agent. The ratio between the amounts of organic phase and seeds influences directly the beads porosity. The lower is that ratio the higher will be the porosity.

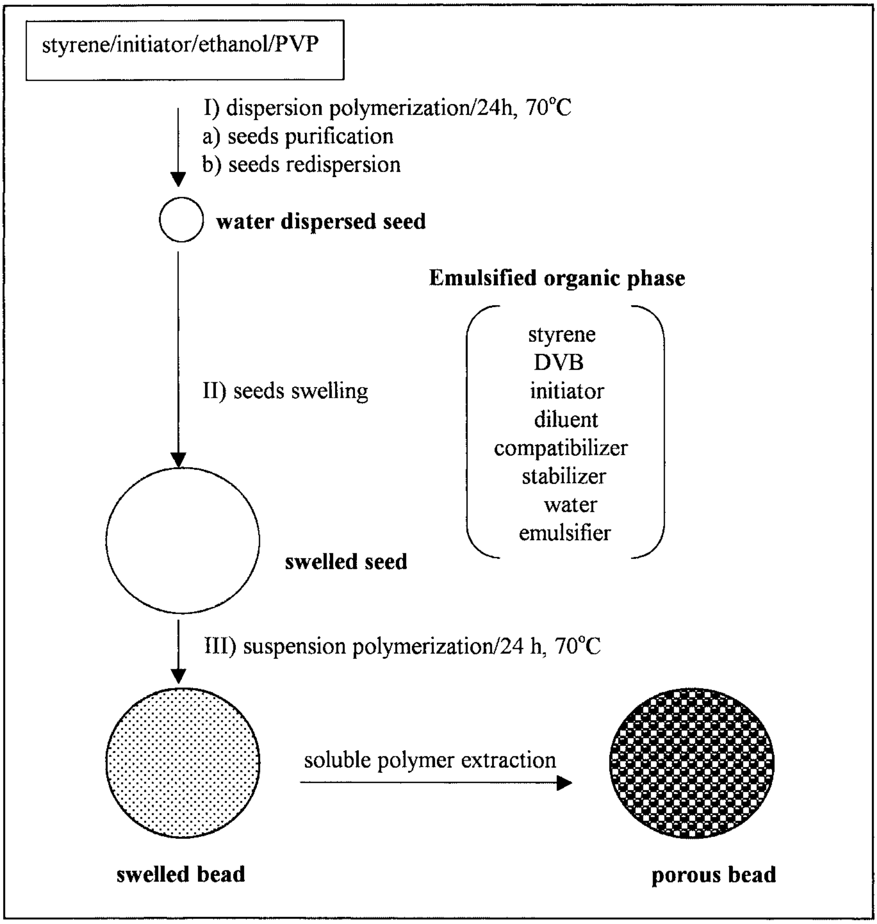


Figure 6: Schematic representation of the porous beads synthesis by SSWP

Chloroform slurries of porous beads were packed, as described previously [21], into stainless-steel columns (30 cm x 7.8 mm i.d.) by a high pressure method to produce SEC columns. The SEC calibration curves were obtained for polystyrene standards using chloroform as eluent at a flow rate of 1.0 mL/min, utilizing a Waters pump (Model 510) equipped with a Waters refractive index detector (Model 410). The upper limit of the range of molecular weight separation (exclusion limit – E.L.) of each column was determined from the calibration curve. The porous structure of the beads define the limits of the separation range and the columns resolution, and the particle size and porosity determine the number of theoretical plates (NTP) also known as efficiency of the column. The average pore diameter (D_i) of the beads in the swelling state in a good solvent is related to the upper limit of the range of molecular weight separation (E.L.) by the equation of Halász[22] ($D_i = 0.62xM^{0.59}$).

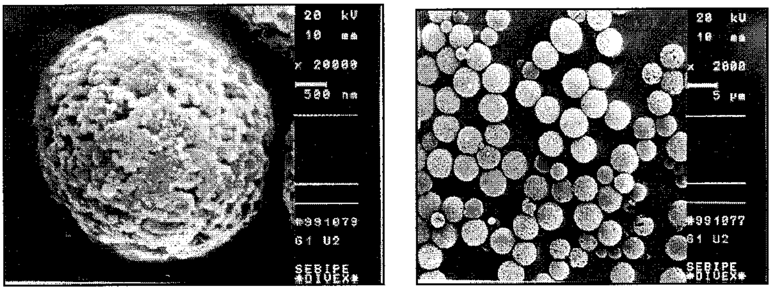
Table 3 shows the conditions to produce, from polystyrene seeds, beads of poly(styrene-co-divinylbenzene) with sizes and distribution of sizes adequate to be applied as packing material in size exclusion chromatography. That table also presents the SEC parameters of the columns packed with those materials.[12,18]

Table 3: SSWP conditions and particle size of poly(styrene-co-divinylbenzene) beads[11]

Seeds code	Copolymer code	OP/seeds w/w	Seed diameter µm	Bead diameter µm	E.L.	NTP	R	D_i Å
PS-34	SSW-07	40	0.94	4.49	$> 10^6$	10900	1.1	2150
PS-34	SSW-08	20	0.94	3.55	$> 10^6$	6100	1.6	2150
PS-10	SSW-10	100	1.73	5.03	10^5	6000	1.7	553
PS-42	SSW-11	100	1.10	3.06	10^5	7500	1.7	553

E.L. = exclusion limit; NTP = number of theoretical plates; R = resolution; D_i = average pore diameter in the swelling state, calculated by the equation $D_i = 0.62xM^{0.59}$, where M is the weight average molecular weight.[22] DVB = divinylbenzene; Sty = styrene; DVB/Sty = 65/35 (molar ratio); toluene/(DVB + Sty) (w/w) = 1/1; isoamyl alcohol = 5% w/w (relative to organic phase); organic phase (OP) = DVB + Sty + toluene; polymerization temperature = 70°C; polymerization time = 10h; stirring speed = 100rpm; initiator = ADVN; [ADVN] = 1% molar (relative to the monomers); aqueous phase = AP; OP/AP (w/w) = 1/10; AP = water (ten times the organic phase weight) + 0.25% (w/w) of sodium lauryl sulphate + 0.6% (w/w) of poly(vinyl alcohol).

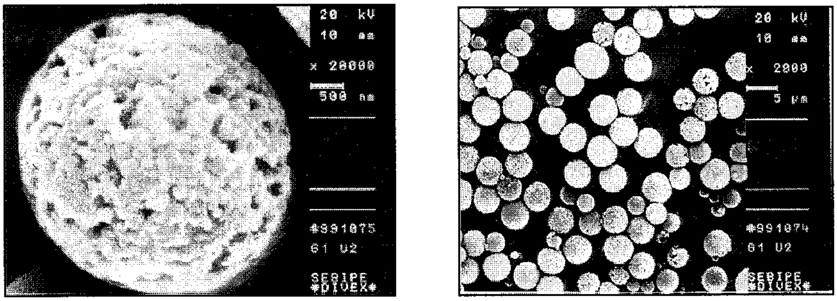
The beads produced with the seeds PS-34 using the lowest OP/seeds ratio (40 and 20) (SSW-07 and SSW-08) (Table 3) presented high porosity, that was evidenced by SEM technique (Figure 7-a and 8-a) and by the high values of exclusion limit ($>10^6$).



(a) (b)

Figure 7: SEM micrographs of poly(styrene-co-divinylbenzene) –SSW-07

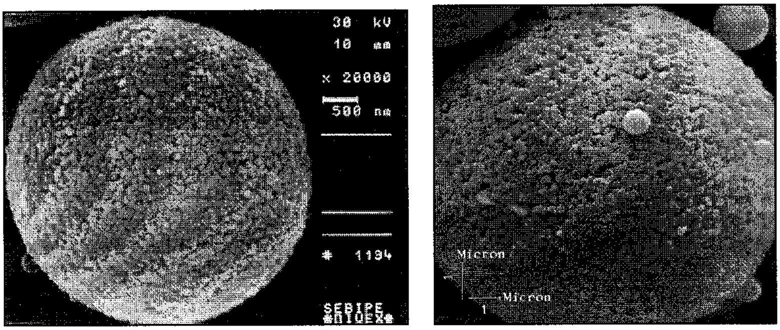
However, SEM micrographs of those beads with lower magnifications (Figures 7-b and 8-b) showed that non-porous beads were also formed. The non-porous beads formation may be attributed to the polymerization of organic droplets that were not absorbed by the polystyrene seeds. As the diluent of the organic phase was toluene, a good solvent for the produced copolymer, non-porous beads, by conventional suspension polymerization (CSP), were formed out of the seeds, the true reactor in SSWP method. The non-porous beads are responsible for the low values of resolution (1.1 and 1.6) of the SEC columns, inferior to the minimum value (1.8) required for an efficient column. The NTP's, which minimum value is around 10000, were also affected by the presence of non-porous beads.



(a) (b)

Figure 8: SEM micrographs of poly(styrene-co-divinylbenzene) –SSW-08

The beads synthesized with the highest (100) OP/seeds ratio (SSW-10 and SSW-11), i.e. the lowest proportion of polystyrene seeds, presented the lowest porosity (Figure 9) and consequently the lowest exclusion limit. This behavior is in accordance with the seeds'role as porogen agent.



(a) (b)

Figure 9: SEM micrographs of poly(styrene-co-divinylbenzene) – (a) SSW-10; (b) SSW-11

Poly(styrene-co-divinylbenzene) beads synthesized by MSP method

The second approach to the synthesis of porous copolymer beads with sizes adequate for application as SEC columns packing materials is the MSP. This suspension polymerization method is preceded by the dispersion of organic phase in the aqueous phase at temperature not exceeding 10°C at a stirring speed of 20,500 rpm during 10 minutes. Afterwards the stirring is slow down to 500 rpm, the reaction mixture is heated to 70°C and maintained at this temperature during the polymerization period (10 hours).[16,17]

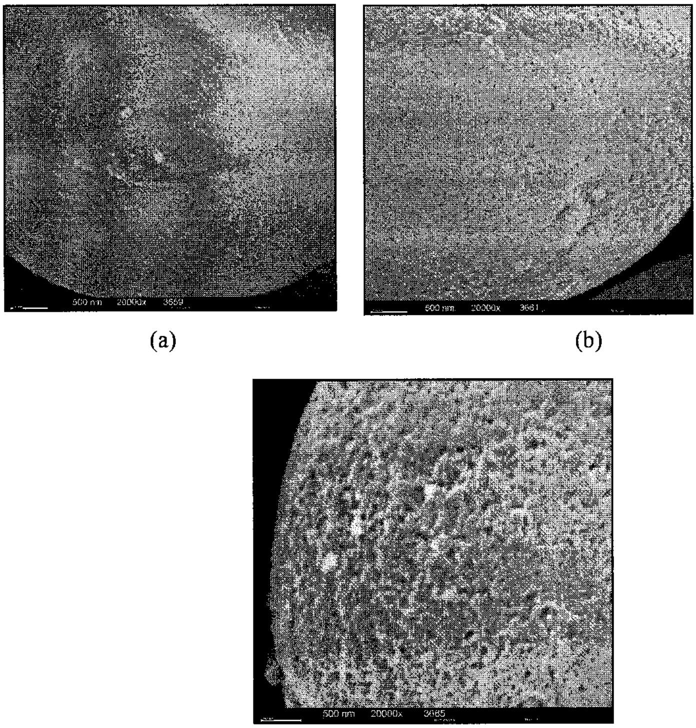
Poly(styrene-co-divinylbenzene) beads were prepared by MSP method employing two different systems as porogen agent: a toluene/heptane (30/70 v/v) mixture and a toluene solution of polystyrene (PS-10; M_w = 146000; M_n = 22000). In the system using toluene/heptane as porogen only the molar ratio DVB/Sty was varied. The average bead diameter became around 10 μm (Table 4). The SEM micrographs of the beads (Figure 10) show that the beads'porosity increased as the DVB content increased. That behavior is in accordance with the mechanism of porosity formation. Higher amounts of DVB (crosslinking agent) provokes the earlier occurrence of macrogelation and consequent phase separation responsible for the pores formation. As expected the SEC columns exclusion limit increased as the DVB proportion increased from 55% to 75%, but did not vary with the posterior increase of DVB from 75% to 100%. This behavior needs to be further investigated because SM-13 (100% DVB) is accentuately more porous than SM-

12 (75% DVB). Probably, this result is due to some problem during the column packing.

Table 4: MSP conditions and particle size of poly(styrene-co-divinylbenzene) beads obtained using as porogen a 30/70 toluene/heptane mixture[a] [12]

Copolymer code	DVB/Sty molar ratio	Bead diameter µm	E.L.	NTP	R	D_i Å
SM-09	55/45	9.34	$1.5\text{x}10^5$	4000	1.1	702
SM-12	75/25	10.55	$4.22\text{x}10^5$	3800	3.8	1292
SM-13	100/0	11.59	$4.22\text{x}10^5$	3700	1.7	1292

E.L. = exclusion limit; NTP = number of theoretical plates; R = resolution; D_i = average pore diameter in the swelling state, calculated by the equation $D_i = 0.62\text{x}M^{0.59}$, where M is the weight average molecular weight.[22] [a]Polymerization initiator = ADVN; [ADVN] = 1%molar (relative to the monomers); pre-stirring time at 10°C = 10 min; polymerization time = 10 h; polymerization temperature = 70°C; porogen = toluene/heptane (30/70 v/v); (toluene + heptane)/(Sty + DVB) = 2/1 (v/v); suspension stabilizer = poly(vinylalcohol) (PVA); aqueous phase (AP) = water + 0.5% w/v PVA; organic phase (OP) = (styrene + divinylbenzene + porogen); AP/OP = 4/1 (v/v); polymerization stirring speed = 500 rpm



(a) (b)

(c)

Figure 10: SEM micrographs of poly(styrene-co-divinylbenzene) – (a) SM-09; (b) SM-12; (c) SM-13

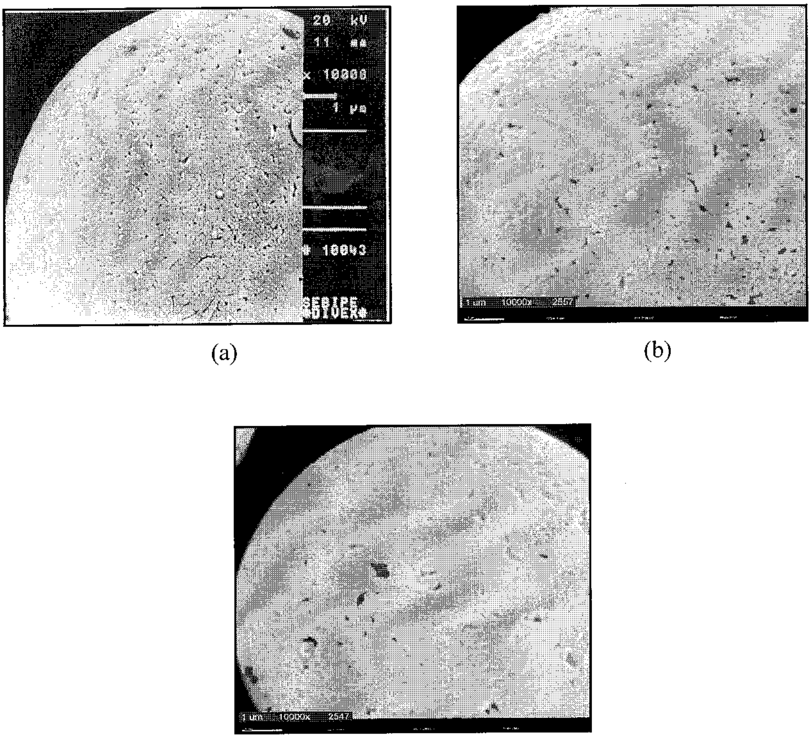
The MSP method using as porogen agent a toluene solution of polystyrene (PS) produced beads with different porosities as the amount of polystyrene or DVB was varied. Using a 6% PS toluene solution and varying the DVB proportion (55%, 75%, 100%) were obtained beads with average diameter around 11 μm and with porosities dependent on the DVB content (Table 5). The SEM micrographs of the beads (Figure 11) show a slight porosity increase as the DVB content increased. It was observed a tendency to higher values of porosity for these beads than for the ones produced in presence of toluene/heptane mixture as porgen agent. The exclusion limit of the SEC columns increased as the DVB proportion increased from 55 to 100%, but remained constant when the DVB amount was increased from 55 to 75%. Probably this result is due to the small difference in porosity of those two copolymer beads.

Table 5: MSP conditions and particle size of poly(styrene-co-divinylbenzene) beads obtained using as porogen a 6% PS toluene solution[a] [12]

Copolymer code	DVB/Sty molar ratio	Bead diameter μm	E.L.	NTP	R	D_i Å
SM-35	55/45	11.1	$4.22x10^5$	6800	1.1	1292
SM-39	75/25	11.8	$4.22x10^5$	3600	1.1	1292
SM-37	100/0	11.3	$8.00x10^5$	4300	1.1	1885

E.L. = exclusion limit; NTP = number of theoretical plates; R = resolution; D_i = average pore diameter in the swelling state, calculated by the equation $D_i = 0.62xM^{0.59}$, where M is the weight average molecular weight.[22] [a]Polymerization initiator = ADVN; [ADVN] = 1%molar (relative to the monomers); pre-stirring time at 10°C = 10 min; polymerization time = 10 h; polymerization temperature = 70°C; porogen = 6% PS toluene solution (w/w); toluene/(Sty + DVB) = 1/1 (v/v); suspension stabilizer = poly(vinylalcohol) (PVA); aqueous phase (AP) = water + 0.5% w/v PVA; organic phase (OP) = (styrene + divinylbenzene + toluene); AP/OP = 4/1 (v/v); polymerization stirring speed = 500 rpm

The largest variations of porosity occurred when the amount of DVB was fixed at 100% and the amount of polystyrene in the porogen solution was varied from 1% to 6% (Table 6). This behavior was translated by the accentuated variation of the exclusion limit of the SEC columns obtained with these beads. The beads synthesized with 1% of porogen produced a SEC column which exclusion limit was $1.02x10^4$ while the column packed with the beads synthesized with 6% of porogen presented a exclusion limit of $8.0x10^5$.



(a) (b)

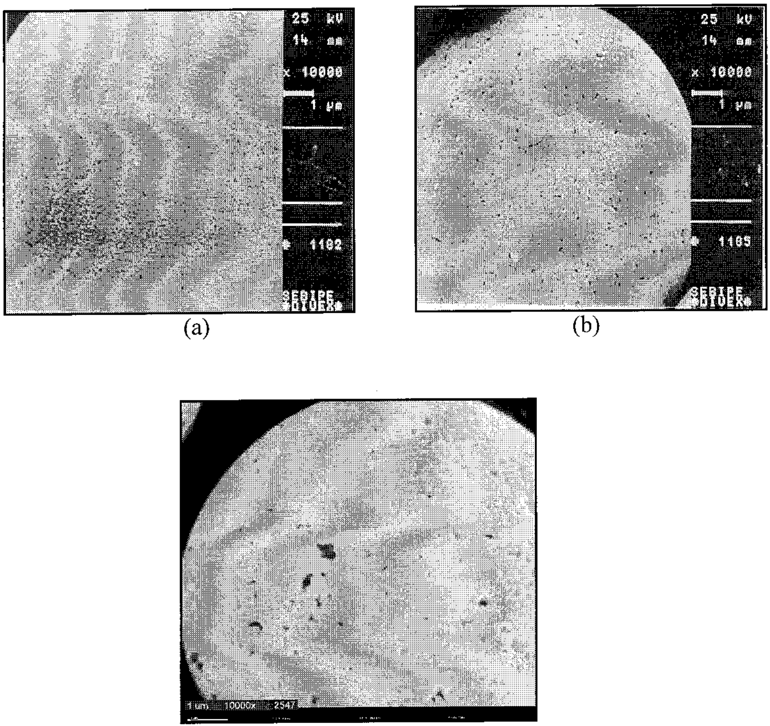
(c)

Figure 11: SEM micrographs of poly(styrene-co-divinylbenzene) – (a) SM-35; (b) SM-39; (c) SM-37

Table 6: MSP conditions and particle size of poly(styrene-co-divinylbenzene) beads obtained using as porogen PS toluene solutions at 100% DVB[a] [12]

Copolymer code	PS/toluene w/w %	Bead diameter μm	E.L.	NTP	R	D_i Å
SM-23	1	11.1	$1.02x10^4$	900	1.5	144
SM-24	4	9.9	$1.42x10^5$	1700	1.0	680
SM-37	6	11.3	$8.00x10^5$	4300	1.1	1885

E.L. = exclusion limit; NTP = number of theoretical plates; R = resolution; D_i = average pore diameter in the swelling state, calculated by the equation $D_i = 0.62xM^{0.59}$, where M is the weight average molecular weight.[22] [a]Polymerization initiator = ADVN; [ADVN] = 1%molar (relative to the monomers); pre-stirring time at 10°C = 10 min; polymerization time = 10 h; polymerization temperature = 70°C; DVB = 100%; toluene/(DVB) = 1/1 (v/v); suspension stabilizer = poly(vinylalcohol) (PVA); aqueous phase (AP) = water + 0.5% w/v PVA; organic phase (OP) = (divinylbenzene + toluene); AP/OP = 4/1 (v/v); polymerization stirring speed = 500 rpm



(a) (b)

(c)

Figure 12: SEM micrographs of poly(styrene-co-divinylbenzene) – (a) SM-23; (b) SM-24; (c) SM-37

Conclusion

1) The highest value of exclusion limit (10^6) for the SEC columns packed with poly(styrene-co-divinylbenzene) beads was attained when the beads were synthesized, employing polystyrene seeds, by the single-step swelling and polymerization (SSWP) method using high proportion of seeds (OP/seeds = 20 or 40).

2) The poly(styrene-co-divinylbenzene) beads prepared by modified suspension polymerization (MSP) method were, in general, less porous than the beads obtained by SSWP method, in equivalent experimental conditions.

3) The most porous poly(styrene-co-divinylbenzene) beads, prepared by MSP method, were those synthesized using polystyrene/toluene solution as porogen agent. The maximum value of exclusion limit attained, in this case, was 8.0×10^5.

4) High proportions of polystyrene, as porogen agent, and DVB produced the SEC columns with the highest values of exclusion limit.

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