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Review

Beaded polymer supports and gels

I. Manufacturing techniques

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ABSTRACT

A general review of preparative aspects of beaded polymer supports and gels (microbeads) is presented. Basic features of manufacturing processes employed for the production of beaded organic and inorganic polymers and gels are discussed. Typical procedures for the synthesis of beaded silica, polysaccharides, polyacrylamides, polymethacrylates and polystyrene are described. Preparative aspects of microspherical products based on inorganic–organic composites, interpenetrating networks, pellicular particles, core–shell grafts and recently introduced amphiphilic copolymer resins are also discussed.

CONTENTS

1. Introduction 182
2. Basic features of two-phase suspension processes 183
    2.1. Definitions and criteria 183
    2.2. Droplet formation. 183
    2.3. Droplet/particle stabilization 184
3. Synthetic organic polymers 184
    3.1. Polystyrene 184
    3.2. Polyacrylamides 186
    3.3. Other synthetic polymers 187
4. Polysaccharides 188
    4.1. Agarose 188
    4.2. Cellulose 189
    4.3. Dextran 189
    4.4. Other polysaccharides 189
5. Inorganic supports 190
    5.1. Granular silica gel 190
        5.1.1. Silica sol 190
        5.1.2. Silica hydrogel 190
        5.1.3. Silica xerogel 190
    5.2. Silica microbeads 191
        5.2.1. Suspension gelation 191
        5.2.2. Suspension polycondensation 191

5.2.3. Microencapsulation 191
5.2.4. Spray drying 191
5.3. Pellicular silica 192
5.3.1. Single-step aerosil coating 192
5.3.2. Multi-step aerosil coating 192
5.3.3. Polycondensation coating 192
5.4. Controlled pore glass 192
6. Composite supports 192
6.1. Pore matrix composites 193
6.2. Interpenetrating networks 193
6.3. Core–shell grafts 194
6.4. Pellicular supports 195
7. Copolymer supports 195
References 195

1. INTRODUCTION

Beaded polymer supports and gels (gel microspheres, microbeads; see Fig. 1) are widely used as packing materials for various chromatographic techniques [1–5]. Microspherical polymer products are also employed for a number of related applications, including biochemical transformations [6], immunology [7], water treatment [8] and the extraction of precious metals [9]. Reviews of the use of polymer supports for solid-phase peptide synthesis, catalysis and other chemical applications are cited in Part II [10].

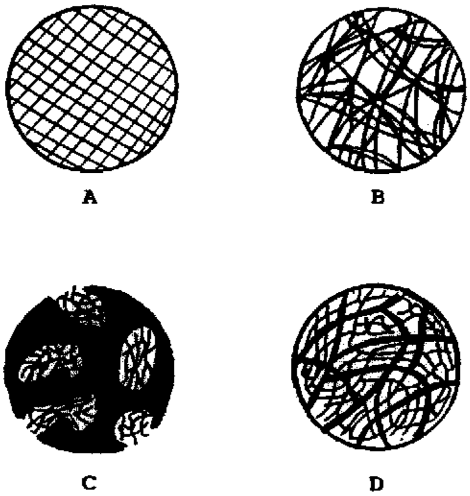


Fig. 1. Schematic presentation of typical particle morphologies of beaded polymer supports and gels used for chromatography. A = Homogeneous matrix (isoporous); B = heterogeneous matrix (macroporous); C = pore–matrix composite; D = interpenetrating network.

Beaded polymer supports and gels are produced from a wide range of natural and synthetic sources (Fig. 2) and by numerous manufacturing processes and technologies. For example, beaded polystyrene resins are produced by oil-in-water (o/w) suspension polymerization, whereas polyacrylamide gels are obtained by water-in-oil (w/o) suspension polymerization. Microspherical poducts based on polysaccharides and silica are manufactured by a number of

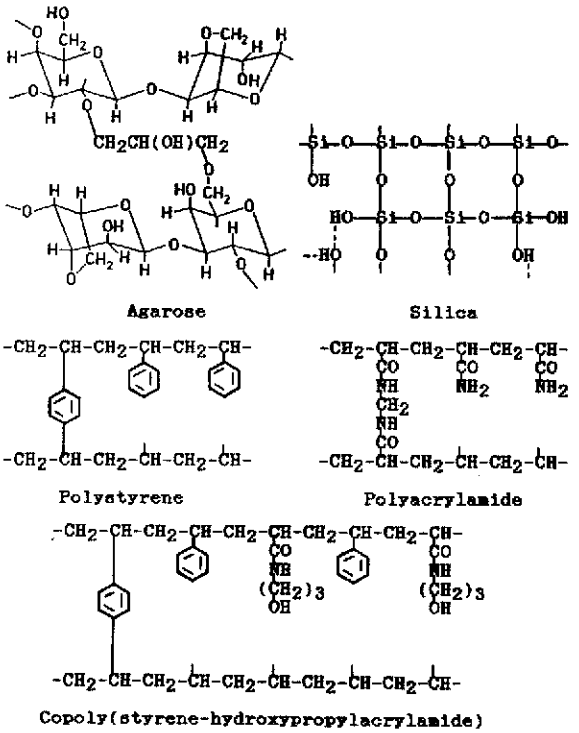


Fig. 2. Structures of different types of beaded polymer supports and gels.

related processes, the details of which are not frequently reported in the literature. In either case, manufacturing parameters are controlled to obtain the beaded product with a relatively narrow particle size distribution within the range of about 5–500 μm. A variety of particle morphologies may also be produced, as indicated in Fig. 1. The products thus obtained may be hydrophilic or hydrophobic, gelatinous or rigid, or they may have low or high porosity and surface area, depending on their chemical structure, degree of cross-linking and manufacturing conditions. Following their production, the microbeads may carry reactive groups, or they may be derivatized and activated by an extensive array of synthetic routes and reagents.

Despite widespread use of microspherical polymer products as packing materials for chromatography [1–5], systematic coverage of the preparation of these materials is lacking in the literature. This two-part review is therefore intended to provide a general picture of the manufacturing aspects of beaded polymer supports and gels. Part I discusses various polymerization and particle formation processes employed for the manufacture of both inorganic and organic polymer and gel microbeads. It covers the preparation of all major packing materials, including silica, polysaccharides, polyacrylamides, polymethacrylates and polystyrene, and also composites and copolymers. Chemical structure, derivatization and the control of physicochemical criteria (*i.e.* particle size, swelling behaviour, porosity and surface area) are discussed in Part II.

It is hoped that this two-part discussion will stimulate a better appreciation of the behaviour and performance of microspherical packing materials on the basis of their chemical structure and synthetic methodology. It should also provide a useful guide and source information for those who may be interested in preparing tailor-made microbeads in the laboratory. For this purpose, a full coverage of more recently introduced beaded polymers, copolymers and composites is provided.

2. BASIC FEATURES OF TWO-PHASE SUSPENSION PROCESSES

2.1. *Definitions and criteria [11,12]*

Beaded polymer supports, whether silica, agarose, cellulose, polyacrylamide or polystyrene, are all produced by different variations of two-phase suspension processes. Therefore, it is instructive here to define the term "suspension process" as a two-phase system in which "liquid microdroplets" are converted to the corresponding "solid microbeads".

Suspension polymerization of water-insoluble monomers (*e.g.* styrene and methyl methacrylate) involves the formation of a droplet suspension of the monomer in water and direct conversion of the individual monomer droplets into the corresponding polymer beads. Preparation of beaded polymers from water-soluble monomers (*e.g.* acrylamide) is similar, except that an aqueous solution of the monomer is dispersed in an oil to form a water-in-oil (w/o) droplet suspension. Subsequent polymerization of the resulting monomer droplets produces the corresponding swollen polyacrylamide beads. This process is often referred to as inverse suspension polymerization, but this terminology should not be used because it implies a restrictive definition of the term "suspension". Beaded silica gel is produced by, *inter alia*, suspension polycondensation and suspension gelation, while polysaccharide-based polymer supports are mostly obtained by suspension gelation and suspension cross-linking.

Among the various suspension systems mentioned above, the details of o/w suspension polymerization are more fully known [13–15]. Accordingly, the following discussion is based on this system. However, it will become abundantly clear throughout the subsequent sections that the criteria of "droplet formation", "droplet stabilization" and "droplet hardening", as discussed below for o/w suspension polymerization, apply equally to the preparation of beaded polymer supports in general.

2.2. *Droplet formation*

The most important feature of o/w suspension polymerization is the formation of a droplet suspension of the monomer in water (the suspension medium) and the maintenance of the individual droplets throughout the polymerization process. Droplet formation in an oil–water mixture is most appropriately accomplished by mechanical stirring, although other forms of mixing can also be employed. For most practical purposes, the volume ratio of the monomer phase to water is smaller than unity (usually 1:10–1:2), although stable suspensions with o/w ratios of > 1 can also be produced.

In a non-polymerizing suspension system [16], the suspended droplets collide with each other, coalesce into larger ones and rapidly redivide into smaller ones again. Under these conditions the system is in a state of dynamic equilibrium and remains apparently stable upon continued mixing. In a polymerizing suspension system, although the same principles apply initially, redivision of the coalesced (larger) monomer droplets becomes gradually more difficult as a result of polymerization and increased viscosity. This means that at a certain stage of the polymerization (beginning of the sticky period), re-division of the partially polymerized droplets becomes almost impossible and continued droplet coalescence leads to coagulation of the entire bulk of the monomer phase. However, assuming that the individuality of the partially polymerized droplets can be maintained by one means or another, progress of the polymerization reaction leads to gradual hardening of the droplets. Again at a certain stage (end of the sticky period), the hardened droplets will no longer coalesce in the event of any collision. The period during which the partially polymerized droplets can combine but not redivide is termed the sticky period, and is usually observed between 25 and 75% conversion, depending on the nature and composition of the monomer mixture.

2.3. Droplet/particle stabilization

Mass coagulation during the sticky period can be prevented by reducing the surface tension of the droplets and minimizing the force with which they collide. The latter is a matter of apparatus design (principally of the distribution of the stirring force), and the former is achieved by using a small amount of a suitable droplet stabilizer (suspension agent or coagulation inhibitor).

In o/w suspension polymerization, the addition of a small amount of a water-insoluble inorganic salt to the suspension system causes the formation of a very thin film around the monomer droplets and thus greatly reduces the danger of coagulation. Organic polymers which are insoluble in the monomer droplets and have relatively low solubility in the suspension medium are also highly effective as droplet stabilizers. Organic polymers are usually preferred to insoluble inorganic salts because they are more easily removed from the surface of the beads by aqueous stripping.

Examples of inorganic droplet stabilizers used for o/w suspension polymerization include talc, bentonite, calcium sulphate and calcium orthophosphate. Among the most commonly used organic stabilizers for o/w suspension systems are polyvinylpyrrolidone and poly(vinyl alcohol) (75–98% hydrolysed). A wide range of other water-soluble polymers such as methylcellulose, gelatin, acacia and other natural gums are also used. In general, a relatively low concentration of the stabilizer (*ca.* 0.1–1%)) is sufficient to maintain a stable suspension system under constant stirring conditions. It is noteworthy, however, that droplet stabilization is a surface phenomenon. Therefore, the minimum stabilizer concentration required for a full monolayer coverage of the droplets increases with decreasing particle size.

The relationship between particle size and the concentration of droplet stabilizer and other manufacturing parameters will be discussed in Part II. Here it should be mentioned that the use of unnecessarily high concentrations of stabilizer should be avoided, because they lead to increased monomer solubilization, lower yields and poorer quality of the beaded polymer product. In practice, both the type and concentration of stabilizer are arrived at empirically. Similarly, the design of the suspension polymerization reactor plays an important role in the stabilization of the suspension system and the quality of the beaded polymer product. Fig. 3 shows a cylindrical suspension polymerization reactor vessel especially designed [13,17] for laboratory preparation of beaded polymer supports.

3. SYNTHETIC ORGANIC POLYMERS

Synthetic organic polymers used as polymer supports or polymeric reagents are mainly based on polystyrene and polyacrylamides. Polymethacrylates and poly(vinyl alcohol) are also used to a lesser extent. Beaded polycondensates such as phenolic and polycarbonate resins can be produced readily by suspension polycondensation [18], but polycondensates are generally less suitable for the development of polymer supports.

3.1. Polystyrene

Styrene-based polymer supports are produced by o/w suspension copolymerization of styrene and

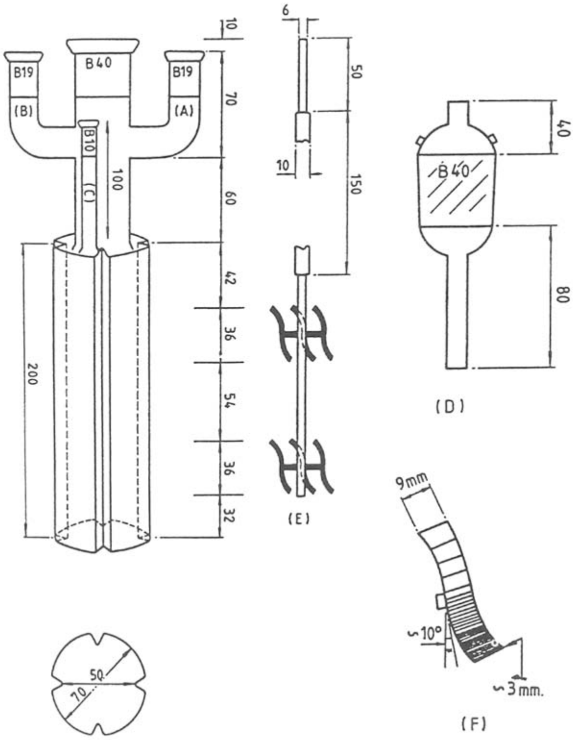


Fig. 3. Laboratory-scale suspension polymerization apparatus. A and B = accession points for a reflux condenser and nitrogen inlet; C = sampling arm; D = a stirrer guide; E = stirrer with its position indicated for full-scale operation; F = expanded drawing of a single stirrer blade indicating the curvature at both ends and the position and angle of attachment to the stirrer rod. All dimensions are in mm.

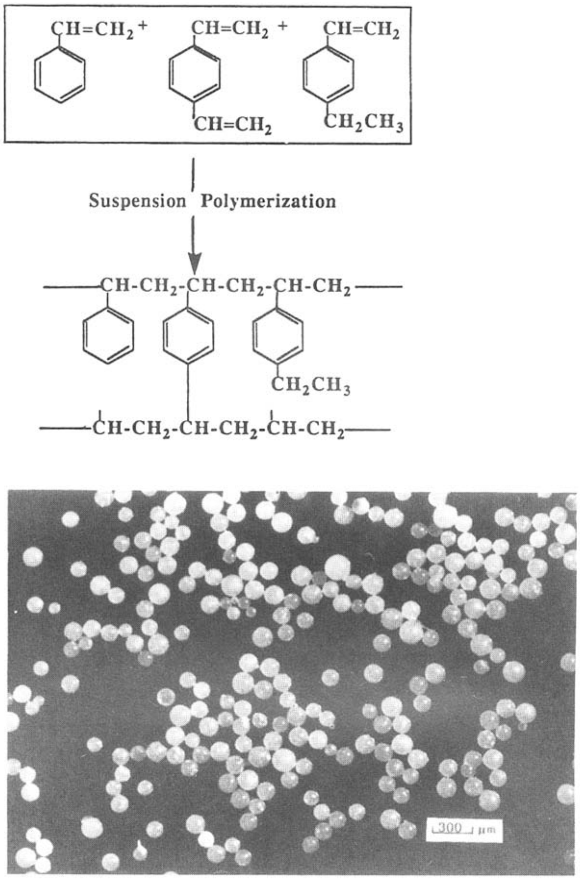


Fig. 4. Synthesis of styrene-based polymer supports by copolymerization of styrene, divinylbenzene and ethylvinylbenzene.

divinylbenzene (DVB), as indicated in Fig. 4. The DVB monomer commonly used for this purpose is a technical mixture composed of divinylbenzene isomers (*ca.* 60%, mainly *meta* and *para*), ethylvinylbenzenes (*ca.* 36%) and small percentages of related aromatic compounds. Suspension polymerization is usually carried out by using a monomer-soluble initiator such as benzoyl peroxide (BP) or 2,2′-azobis-2-methylpropionitrile (AIBN), at a temperature of 60–80°C. A relatively high initiator concentration [1–2% (w/w) based on monomer] is used, and the polymerization is allowed to proceed to completion (98–100%). The time required for complete monomer conversion must be determined by preliminary experiments, but is usually between 5 and 15 h, depending on the initiator concentration, temperature and the exact composition of the monomer mixture (see Table 1).

In addition to the monomers and the initiator, an inert liquid (referred to as a monomer diluent or porogen) may also be added to the monomer phase to influence the pore structure and swelling behaviour of the beaded resin product. The monomer diluent (not to be confused with the water used as the suspension medium) is usually a hydrophobic liquid such as toluene, dodecane or pentanol (see Table 1). These liquids represent examples of good, poor and non-solvents, respectively, for polystyrene, and their use leads to the formation of isoporous, microporous or macroporous beads [27,28], as discussed in Part II.

It is noteworthy that the nature and percentage of

TABLE 1

TYPICAL PROCEDURES FOR OIL-IN-WATER (O/W) SUSPENSION POLYMERIZATION REPORTED FOR THE PREPARATION OF BEADED COPOLYMERS OF STYRENE AND DIVINYLBENZENE

Monomer diluent	o/w vol. ratio	Droplet stabilizer[a]	Remarks	Ref.
Toluene, xylene or diphenylmethane	1:2	A	Early synthesis and study of porous resins	19
Toluene, dodecane or isoamyl alcohol	?	?	Synthesis and evaluation of porous resins for gel permeation chromatography	20
None or chlorobenzene	1:10	B	Functionalized polymer supports	21
None	1:5	A	Systematic study of suspension polymerization	22
None	?	C	Reduction in the amount of emulsified polymer	23
Ethylhexanoic acid	1:1	D	Study of pore formation	24
Hexane–toluene	1:4	E	Study of pore formation	25
None	1:1.5	F	Chloromethylated supports	26

[a] A = Poly(vinyl alcohol); B = a mixture of polyvinylpyrrolidone, calcium orthophosphate and calcium sulphate; C = hydrophobically modified polyethylene glycol or hydroxyethylcellulose; D = copoly(styrene maleic acid) ammonium salt; E = bentonite and gelatin; F = gelatin and poly(allyldimethylammonium chloride).

the monomer diluent also influence the rate of polymerization. This may be mainly a concentration or precipitation effect, depending on whether the diluent is a solvent or precipitant for the polymer. For example, when the diluent is a good solvent, such as toluene or chlorobenzene, the polymerization proceeds at a correspondingly slower rate, whereas with a non-solvent such as pentanol the opposite is true.

Following the completion of the polymerization process, the beaded polymer product is recovered from the suspension mixture and freed from the stabilizer, diluent and traces of monomers and initiator. In industry, this is usually accomplished by steam stripping [29,30]. For laboratory preparations, repeated washing with water, a chlorinated solvent (*e.g.* chloroform) and methanol, respectively, is more appropriate [13]. Complete removal of the monomer diluent, especially from macroporous resins, may require long equilibration times with methanol, and some workers use a Soxhlet apparatus at the final stage of the recovery process.

Monodisperse polystyrene beads are produced by a special mode of seeded polymerization developed by Elingsen *et al.* [31]. According to this method, an aqueous polystyrene latex is equilibrated, first with a suitable oligomer and then with styrene and DVB. Under carefully controlled experimental conditions, the monomers are absorbed uniformly by the seed latex particles. Subsequent polymerization of the swollen particles (*i.e.* monomer droplets in the aqueous medium) produces the corresponding polymer beads. The size and cross-linking of the resulting particles are determined by the amount of the monomers used for swelling. This method is generally applicable, but is especially useful for producing uniform particles in the range 2–20 μm.

Beaded polystyrene resins can also be produced by suspension cross-linking of preformed soluble polystyrene. For example [32], a solution of the polymer, the cross-linking agent, 3,6-dimethyl-1,4-dichloromethylbenzene and a Friedel–Crafts catalyst ($SbCl_5$) in dichloroethane is dispersed in silicone oil (oil-in-oil suspension). The polystyrene droplets obtained in this way are then converted to the corresponding swollen polymer beads by effecting the cross-linking reaction at 70°C. This procedure is basically similar to those used for the preparation of beaded polysaccharides, as will be discussed in section 4.

3.2. *Polyacrylamides*

Literature on the synthesis of acrylamide resins for chromatography can be traced back to the late 1950s [33]. Early workers [33–37] produced bulk cross-linked polymers by homogeneous copolymerization of acrylamide and methanediacrylamide (methylenebisacrylamide, Bis), followed by grinding of the bulk polymer to obtain small gel particles. Hjerten and Mosbach [38] passed the swollen acryl-

TABLE 2

TYPICAL PROCEDURES REPORTED FOR THE SYNTHESIS OF BEADED POLYACRYLAMIDES BY WATER-IN-OIL (W/O) SUSPENSION COPOLYMERIZATION OF ACRYLAMIDES WITH BISACRYLAMIDE

Beaded polymer	Monomer diluent	Oil phase	Droplet stabilizer[a]	Remarks	Ref.
Polyacrylamide (Bio-Gel)	0.1 *M* sodium acetate	1,2-Dichloroethane	CAB	Early example of beaded hydrophilic gels	39
	Water	Toluene	A	Use of methacrylamide and hydroxymethylacrylamide	40
	0.1 *M* sodium acetate	Chlorobenzene	CAB	Alternative cross-linking monomers	41
Polyacryloylmorpholine (Enzacryl gel)	Water	Liquid paraffin	Span 85	Resins for gel permeation chromatography	42
Polyacryloyltrihydroxymethylacrylamide (Trisacryl gel)	Water	Petroleum	?	Resin for enzyme immobilization	43
Polydimethylacrylamide (Pepsyn)	Water–DMF[b]	1,2-Dichloroethane	CAB	Functional resins for peptide synthesis and catalysis	44, 45
	Water	?	CMC	Resin for gel permeation chromatography	46, 47

[a] CAB = Cellulose acetate butyrate; A = calcium stearate and dodecylphenoxypolyethoxyethanol; CMC = carboxymethylcellulose.
[b] DMF = Dimethylformamide.

amide gel through a pressurized jet to obtain a granular product. However, in a patent issued in 1964, Flodin [39] described a suspension polymerization procedure for the preparation of beaded acrylamide resins, which has remained the basis of much of the later work in this area.

Beaded acrylamide resins are generally produced by w/o suspension polymerization. This involves the dispersion of an aqueous solution of the monomer and an initiator (*e.g.* ammonium peroxodisulphate) in an immiscible liquid (the oil phase, see Table 2). A polymerization catalyst, usually N,N′-tetramethylethylenediamine (TEMED) or riboflavin, may also be added to the monomer mixture. Under these conditions, the polymerization of most acrylamides proceeds at substantially faster rates than that of styrene in o/w suspension polymerization. For this reason, preparation of beaded acrylamide resins is carried out at relatively low temperatures (20–50°C), and the polymerization is complete within relatively short periods (1–5 h). All of these conditions reduce the risk of droplet coagulation during the suspension polymerization. Accordingly, the problem of droplet coagulation during the synthesis of beaded polyacrylamides by w/o suspension polymerization is less critical than that of styrene-based resins.

Recently Patel *et al.* [48] described an interesting method for the preparation of beaded polyacrylamide gels involving droplet formation by a vibrating needle. Thus, the aqueous monomer solution (containing the initiator and catalyst, TEMED) is dropped into the suspension medium (chlorobenzene–xylene) from an 18-gauge needle at a rate of 0.5–7 g/min. The use of a catalyst and a temperature of 75°C leads to a relatively high rate of polymerization. Under these conditions, the polymerization is complete probably within 5–10 min, and the polymer beads (300–1000 μm) settle at the bottom of the polymerization tube. This procedure provides a particularly versatile method for producing small amounts of polymer, and is also adaptable for large-scale preparations.

3.3. Other synthetic polymers

In an early publication, Heitz *et al.* [49] described the preparation of beaded poly(methyl methacrylate) cross-linked with ethanedimethacrylate, and poly(vinyl acetate) cross-linked with butanediol divinyl ether. The latter polymer is the basis of the Merckogel series of gel permeation chromatographic packings, and its hydrolysed derivative, poly(vinyl alcohol), is marketed as Fractogel and Toyopearls. Beaded methacrylic polymers, poly(hydroxyethyl methacrylate) (Spheron, Separon) [50] and poly(glycidyl methacrylate) (Eupergit) [51,52], have been introduced and extensively studied at the Czechoslovak Academy of Macromolecular Sciences. The most recent addition to the range of beaded polymer supports is poly(oligooxyethylene dimethacrylate)s, described by Trijasson *et al.* [53].

4. POLYSACCHARIDES

Beaded polysaccharide resins (gels) are produced by a variety of closely related suspension processes, one example of which for non-cross-linked cellulose beads is shown schematically in Fig. 5. Most of the techniques developed for the preparation of beaded polysaccharide resins are based on droplet formation processes more or less similar to w/o suspension polymerization, but they differ in the conversion of the droplets to the corresponding solid particles. This may involve a simple gelation or chelation process, solvent extraction, covalent cross-linking, or in the case of allyldextran, suspension copolymerization with bisacrylamide. Representative examples of different processes used for the preparation of beaded polysaccharide gels are described below.

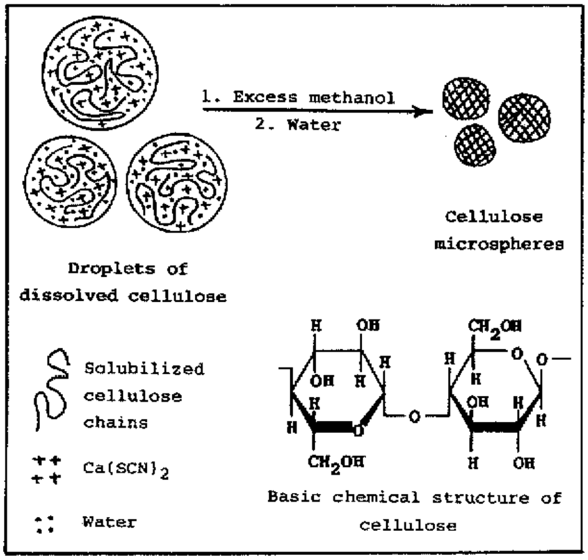


Fig. 5. Schematic presentation of solvent extraction process used for the preparation of beaded cellulose.

TABLE 3

PREPARATION OF AGAROSE BEADS BY SUSPENSION GELATION (ADAPTED FROM REF. 56)

Agarose in 300 ml of solution (g)	Oil phase, toluene-CCl_4 (ml)	Droplet stabilizer (g)[a]	Stirrer speed (rpm)	Bead size (US mesh)
3	490–110	SSO (0.5)	250	30–60
9	470–130	SSO (0.5)	1150	100–170
18	450–150	SSO (1)	1700	100–170
18	450–150	SSO (1.5)	1700	170–300
24	455–155	PSO (5)	1500	60–100
24	455–155	PSO (12)	1700	170–300
30	440–160	PSO (15)	1700	170–300
45	420–180	PSO (35)	1700	170–300

[a] SSO = Sorbitan sesqui-oleate; PSO = polyethylene oxide derivative of sorbitan monooleate.

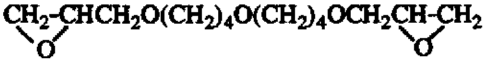
4.1. *Agarose*

Agarose is obtained from a variety of marine algae [54,55]. It is a linear alternating copolymer of (1→3)-β-D-galactopyranose and (1→4)-3,6-anhydro-α-L-galactopyranose, and usually contains a small percentage of sulphate groups (<0.2% sulphur). Agarose is soluble in water at above 50°C up to a concentration of 20% or more, and the solution gels readily when cooled to room temperature. This favourable sol–gel behaviour is the basis of a relatively simple process for the preparation of non-cross-linked beaded agarose (Sepharose), as described by Hjerten in 1964 [56].

Thus, an aqueous solution of 1–5% agarose is stirred in a mixture of toluene and carbon tetrachloride at 50°C, in the presence of a droplet stabilizer. The resulting droplet suspension is then cooled to room temperature under continuous stirring to effect gelation, and hence the formation of non-cross-linked agarose beads (*cf.* Sepharose B). Table 3 provides quantative details of a series of beaded agarose gels obtained according to this procedure [56].

For the preparation of cross-linked agarose beads (*cf.* Sepharose CL-B), the non-cross-linked gels described above are treated with epichlorohydrin, diepoxides, 2,3-dibromopropanol or divinyl sulphone at high pH and 60°C for 2 h. The resulting cross-linked beads are then desulphatized at a higher temperature (120°C). These reactions are usually carried out in the presence of small amounts of a reducing agent (sodium borohydride) to avoid oxidative degradation of the polymer [57,58].

More recently, agarose gels containing two types of cross-linking have been described [59]. Thus, the aqueous agarose droplets are prepared as described above. The w/o droplet suspension is treated with a diepoxide, *e.g.*



$$\underset{\diagdown O \diagup}{CH_2\text{-}CH}CH_2O(CH_2)_4O(CH_2)_4OCH_2\underset{\diagdown O \diagup}{CH\text{-}CH_2}$$

which is soluble in the organic phase, followed by reaction with epichlorohydrin. This cross-linking procedure probably results in the formation of microcapsular particles in which the outer shells are more hydrophobic and more highly cross-linked than the inner cores.

4.2. Cellulose

Cellulose has long atrracted interest as a suitable column packing material for liquid chromatography and other chromatographic separations. However, conventionally produced cellulose powders (known as microcrystalline cellulose) consist of irregularly shaped fibrous particles, and as such do not meet the requirements of modern chromatographic techniques.

Regularly sized cellulose microspheres (referred to as regenerated cellulose) are produced by dissolution of cellulose powder in a suitable solvent (see below), followed by droplet formation in a suspension medium, and subsequent solvent extraction or covalent cross-linking.

Early examples of the preparation of cellulose beads by solvent extraction are those described by O'Neill and Reichart [60] and Determan and Wieland [61]. In the last example, linter cellulose is dissolved in Schweitzer solvent (aqueous ammonium cuprate) and the solution is then stirred in benzene in the presence of a suspension stabilizer (Emulphor EL) to form the desired aqueous cellulose droplets. Addition of the resulting suspension mixture to a large volume of benzene containing acetic or benzoic acid results in solvent extraction, and hence the formation of the corresponding cellulose beads.

Two more recent reports on the preparations of non-cross-linked cellulose beads, by Peska *et al.* [62] and by Kuga [63], are also based on solvent extraction procedures. In the latter work, cellulose (Whatman CF-1, DP 180 or cotton linter, DP 1620) is dissolved in a highly concentrated solution of calcium thiocyanate at 120–140°C. The polymer solution is then stirred in dichlorobenzene at above its gel point (*ca.* 80°C). The resulting droplet suspension is added to cold methanol to effect solvent extraction and to form the corresponding cellulose beads as indicated in Fig. 5. If the dichlorobenzene suspension is prepared below the gel point of the cellulose solution, only irregular cellulose particles are obtained. Fig. 6 shows photomicrographs of both spherical and irregular cellulose particles produced from a 6% solution of cellulose CF-1.

Preparation of cross-linked cellulose beads by suspension cross-linking has been studied by Chitumbo and Brown [64]. In this work, a viscose solution (9.2% cellulose, 7.5% sodium hydroxide and 28% carbon disulphide) is vigorously stirred in dichloroethane containing a suitable suspension stabilizer (*e.g.* Cremophor EL) and a small percentage of sodium borohydride. The temperature of the droplet suspension is brought to 40°C, epichlorohydrin is added, and the stirring is continued until the desired degree of cross-linking is reached (14 h).

4.3. Dextran

Dextran, a branched homopolymer of D-glucose, lacks the structural rigidity which is the basis of the favourable sol–gel transformations in agarose and cellulose. Accordingly, all dextran-based polymer supports are cross-linked. Preparation of Sephadex [65] by cross-linking of dextran with epichlorohydrin or 2,3-dibromopropanol is similar to that of Sepharose, as described above. In the case of Sephacryl [66], bead formation is accomplished by w/o suspension copolymerization of allyldextran with bisacrylamide (*cf.* section 3.2).

4.4. Other polysaccharides

The preparation of cyclodextrin beads has been described in some detail by Fenyvesi *et al.* [67]. The cross-linking agent used in this work is a diepoxide similar to that used in the two-stage cross-linking of agarose beads [59]. Epichlorohydrin cross-linked starch microspheres [68] and bisacrylamide cross-linked acrylstarch microspheres [69] are produced in basically the same way as those of Sephadex [65] and Sephacryl [66], respectively. Chibata *et al.* [70] have

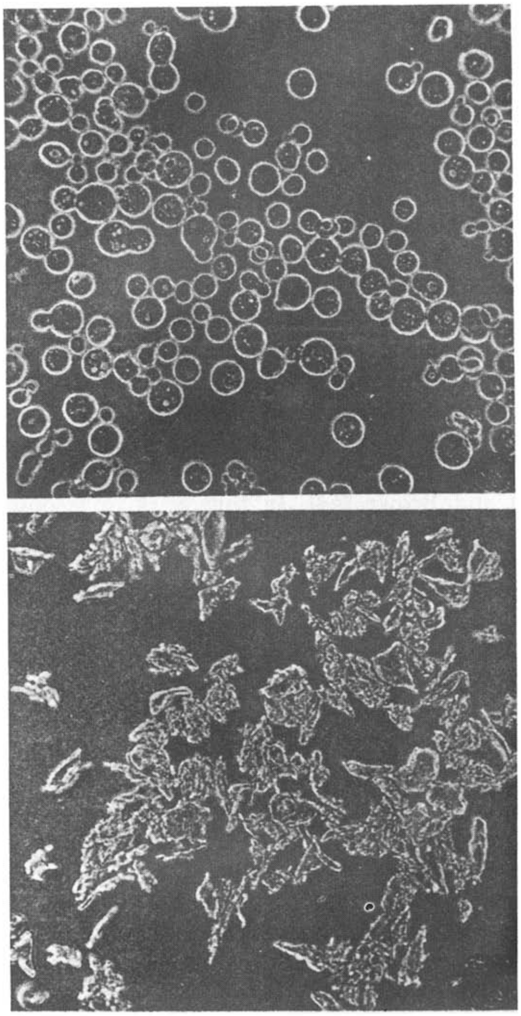


Fig. 6. Phase contrast micrograph of beaded and irregular particles of cellulose produced by solvent extraction (from ref. 63).

described the preparation of κ-carrageenan beads by suspension cross-linking with epichlorohydrin in a fashion similar to that described for agarose [56,57].

5. INORGANIC SUPPORTS

Porous silica (silica gel) is widely used as a sorbent, catalyst and polymer support. Related sorbents such as alumina, titania and zirconia are also used to a lesser extent. Controlled pore glass (CPG) has attracted considerable interest in recent years for enzyme immobilization and affinity chromatography. Zeolites are also extensively used as catalysts and sorbents, but do not fall within the scope of this review. This section discusses the production technology of porous silica in general, and those of microspherical and pellicular silica supports in particular. A brief section is also devoted to CPG for the sake of completeness.

5.1. *Granular silica gel*

The term "silica" in the specific sense refers to SiO_2 as a stoichiometric compound. However, silica-based supports to be discussed here contain various proportions of bound water [*i.e.* $SiO_2(H_2O)_x$]. Granular silica is produced by the conventional sol–gel process, as outlined in Fig. 7. The process involves the formation of a series of silica intermediates, including sodium silicate solution (obtained from caustic dissolution of sand), silicic acids, "silica sol", "silica hydrogel" and "silica xerogel".

5.1.1. Silica sol. The term "silica sol" is used to describe an aqueous dispersion of nanometre-sized silica particles (10–100 nm, colloidal silica), obtained by controlled polycondensation of silicic acid. Thus, acidification of a solution of sodium silicate leads to polycondensation and the formation of poly(silicic acid)s. As the polycondensation proceeds, precipitation takes place and silica nuclei (or primary particles) are formed. These nuclei subsequently grow to colloidal size, and hence the formation of "silica sol" [71]. This process is reminiscent of dispersion polymerization [11].

5.1.2. Silica hydrogel. The growth of the colloidal silica particles in the silica sol can be controlled by a number of parameters, notably silicate concentration, pH, temperature and stabilization by electrolytes [71]. As the number and size of the particles increase, they coagulate and form larger (micrometre sized) particles as a result of interparticle bonding. The continuation of this process (ageing) gradually leads to mass coagulation, and hence the formation of aqueous silica gel (or silica hydrogel).

5.1.3. Silica xerogel. The bulk hydrated gel produced in the sol–gel process is converted to porous dry silica (xerogel) by thermal dehydration. An interesting observation related to the dehydration process is that when the swelling water in the hydrogel is replaced with an organic solvent, and the latter is removed by heat treatment, relatively more

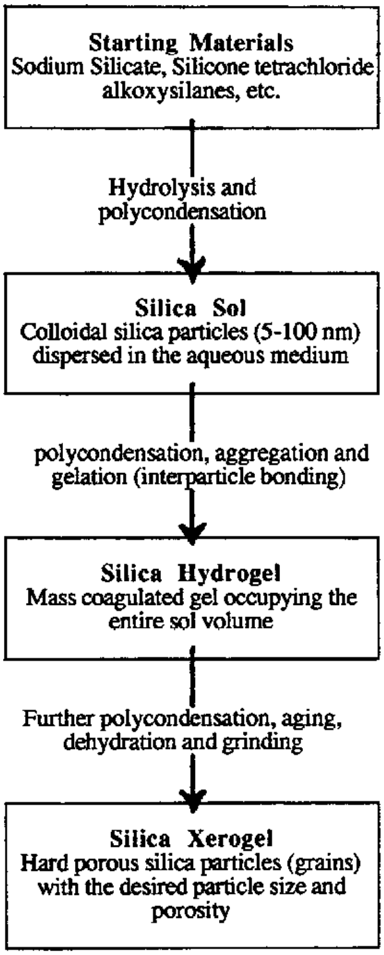


Fig 7. Different steps and intermediates involved in the preparation of irregular silica particles by the conventional sol–gel process.

highly porous particles are obtained [72]. This effect is similar to the diluent effect observed in the synthesis of organic polymer supports in the presence of a monomer diluent (*cf.* section 3.1). The silica gel obtained at the end of the drying stage may be soaked in a salt solution, followed by sintering for further adjustment of particle rigidity and pore structure [73]. The product is finally milled and sieved to produce several particle size ranges (*ca.* 5 μm–5 mm).

5.2. Silica microbeads

The introduction of beaded silica supports (silica microbeads) in the late 1960s and early 1970s [74] followed the popularity of beaded organic polymer supports in chromatography. Accordingly, different suspension systems initially developed for the production of beaded organic polymer supports are also the basis of most of the procedures described for the manufacture of beaded silica. Typical examples of these procedures are outlined below.

5.2.1. Suspension gelation. This procedure is basically similar to the sol–gel process (Fig. 7), except that "gelation" is effected while the silica sol is suspended in the form of small droplets in an organic liquid (suspension medium). Following the sol–gel conversion, the resulting "silica hydrogel beads" are separated, washed, dried and calcined to obtain the corresponding dry silica microbeads [75].

5.2.2. Suspension polycondensation. According to this method [76], a low-molecular-weight polyethoxysilane (PES) is first prepared by partial hydrolysis of tetraethoxysilane. The PES oligomer is then stirred in a water–methanol mixture to form a droplet suspension, followed by addition of a catalyst (*e.g.* ammonia) to effect polycondensation and the formation of the corresponding silica microbeads. This procedure represents an interesting quasi-suspension system in which a single liquid apparently serves both as a solvent (monomer diluent) within the monomer droplets and as a suspension medium in which the droplets are formed.

5.2.3. Microencapsulation. This method involves the entrapment of silica sol particles within an organic polymer matrix, followed by sintering and burning of the organic polymer. For example [77, 78], formaldehyde and urea are added to a well dispersed silica sol, followed by adjustment of the pH to effect the simultaneous polycondensations of silica sol and the organic monomers. This is basically a "dispersion polycondensation" process [18], in which a network of nanometre-sized silica particles are encapsulated within a micronmetre-sized network of the organic polycondensate. The resulting microcapsules are then subjected to heat treatment, first at 500°C to burn the organic polymer, and then at 1000°C to effect a slight sintering of the final silica microbeads.

5.2.4. Spray drying. According to this procedure [79], small droplets of silica sol are sprayed into an oven at 400°C. This results in the evaporation of water and simultaneous polycondensation within

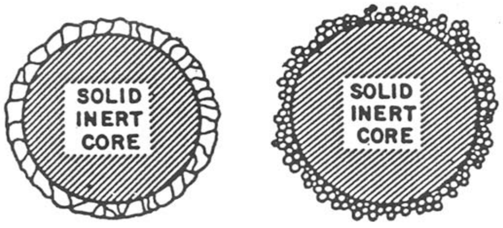


Fig. 8. Schematic presentation of (left) pellicular and (right) superficially porous polymer supports.

the droplets. The semispherical particles obtained in this way are then subjected to a hydrothermal treatment for the adjustment of porosity.

5.3. Pellicular silica

Pellicular and superficially porous silica supports are composed of a compact core and a porous shell or coat, as shown schematically in Fig. 8. The preparation of pellicular silica supports was first introduced by Halász and Horváth in 1964 [80], and a number of modified procedures have since been reported, as outlined below.

5.3.1. Single-step aerosil coating. According to the original method reported by Halász and Horváth [80], clean glass beads are shaken with a dispersion of colloidal silica (Aerosil, 5–50-nm particles) in an organic liquid. The liquid is then removed, and the silica coat is cemented around the glass core by heat treatment.

5.3.2. Multi-step aerosil coating. In this procedure [81], the core particles are first coated with a monolayer of colloidal silica (see above), and then with a positively charged organic polymer such as poly(methacryloxyethyldimethylammonium acetate), followed by drying. This alternating silica–organic coating is then repeated until the desired coat layer thickness (0.5–1 μm) is reached. The coated microbeads are finally subjected to a two-stage heat treatment to burn off the organic polymer and to adjust the pore structure and mechanical stability of the silica coat.

5.3.3. Polycondensation coating. This method [82] involves the formation of a silica layer around the glass particles by direct polycondensation of polyethoxysilane (PES). Thus, cleaned glass beads are dipped in a solution of PES in a low-boiling solvent, followed by evaporation of the solvent. The PES-coated particles are then suspended in a mixture of ethanol and water, and polycondensation of the adsorbed PES is effected by addition of a catalyst (*e.g.* ammonia). The resulting product is allowed to age before washing and drying.

5.4. Controlled pore glass

CPG is obtained from borosilicate glass (SiO_2, B_2O_3, Na_2O) by acid treatment. The process is based on the observation that, on heat treatment, certain borosilicate compositions from two-phase systems in which tiny borate-rich particles exist within a continuous silicate-rich matrix [83]. When this material is treated with acid, the borate phase is leached out and a porous product is thus obtained.

The pores formed by acid leaching are relatively small (30–60 Å). For the preparation of larger pore glasses, the acid leaching is followed by a mild caustic treatment [84,85]. This results in controlled dissolution of silicate from the interior of the pores, and hence the formation of larger pores. The initial phase separation process of borosilicate glass, as well as acid and base treatments, can be carefully "controlled" to produce relatively narrow pore sizes of up to about 3000 Å (300 nm) or larger (Fig. 9). CPG produced in this way typically contains about 95–96% silica glass, 3–5% B_2O_3 and traces of related metal oxides.

6. COMPOSITE SUPPORTS

The variety of polymer supports described in the preceding sections cover a wide range of chemical

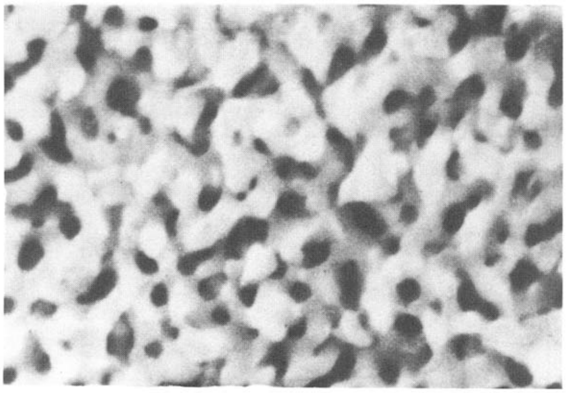


Fig. 9. Scanning electron micrograph of controlled pore glass (magnification 10 500) (adapted from ref. 85).

structures, solvent compatibility, mechanical rigidity and thermal stability. However, most of these polymers have well defined characteristics within relatively sharp boundaries. For example, polystyrene is strongly hydrophobic and is not suitable for aqueous applications, whereas simple polyacrylamide is strongly hydrophilic and not suitable for use in organic media. Some of the more recently introduced products (*e.g.* Enzacryl and Spheron) have intermediate properties, and hence a wider range of applications.

Silica-based supports are rigid (do not swell), but most polyacrylamides and polysaccharides form soft gels in the swollen state. Mechanical rigidity of the support is an important advantage in, for example, high-performance liquid chromatography, especially when a high capacity is not essential. On the other hand, soft gels provide high capacity and increased site accessibility, but they collapse under high pressure.

Most of the supports described here are chemically stable under most operating conditions. However, silica and (to a lesser extent) methacrylate supports are not stable under strongly alkaline conditions. Polysaccharides are prone to degradation by strong acids, oxidants and microorganisms. The methylenebisacrylamide bridges in acrylamide resins are also labile towards strong acids.

In order to benefit from the desirable features of different polymer types, and at the same time minimize their shortcomings, attempts have been directed towards the design of composite and multicomponent polymer supports. The variety of composite polymer supports described in the literature can be divided into four broad categories: (1) pore–matrix composites, (2) interpenetrating networks, (3) cores–shell grafts and (4) pellicular supports.

6.1. Pore–matrix composites

This category of composite supports has attracted considerable interest owing to its simple design and ease of production, as illustrated in Fig. 10. Thus, a porous inorganic support (*e.g.* porous silica or CPG) is soaked in a solution of the organic monomer mixture, usually containing a cross-linker and an initiator. Subsequent polymerization under carefully controlled conditions leads to the formation of the desired organic polymer within the pores of the beaded inorganic support [86–88].

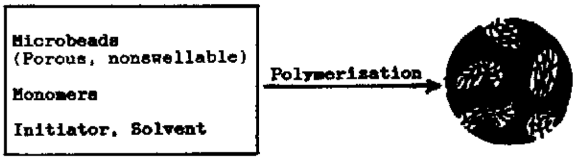


Fig. 10. Preparation of pore–matrix composite supports.

It should be emphasized, however, that the organic polymer must usually be sufficiently cross-linked to be permanently "entrapped" within the pores of the inorganic matrix. Alternatively, a less highly cross-linked organic polymer may be covalently "anchored" to the pore surface. Covalent attachment can be accomplished either by copolymerization with vinyl residues attached to the pore surface [89] or by reaction between appropriately chosen reactive groups on the organic polymer and the inorganic matrix [90].

Another example of pore–matrix type composite supports is that of polyacrylamide–agarose (Ultragel) described by Uriel [91] and Monsigny *et al.* [92]. Here, the composite beads are obtained by suspension polymerization in the same way as polyacrylamide beads (section 3.2), except that the required percentage of agarose is also dissolved in the monomer mixture. Thus, suspension copolymerization of acrylamide and bisacrylamide produces polyacrylamide beads which entrap the agarose chains initially present in the monomer droplets. A scanning electron micrograph of a partially dried Ultragel bead obtained from 4% acrylamides and 4% agarose is presented in Fig. 11 [92]. The micrograph shows a loose network of polyacrylamide rods (*ca.* 100–500 nm thick) and relatively large loops filled with collapsed agarose particles.

6.2. Interpenetrating networks

Beaded polymer supports composed of two interpenetrating networks are produced according to the general scheme shown in Fig. 12 [93]. This method involves the formation of one cross-linked polymer network within the matrix of a preformed beaded polymer. An interesting example of beaded interpenetrating networks is provided by the recently reported molecular organometallic composites [94]. Fig. 13 shows an X-ray microprobe image of a copolymer of vinylferrocene formed within a beaded polydimethylacrylamide matrix [94]. This image

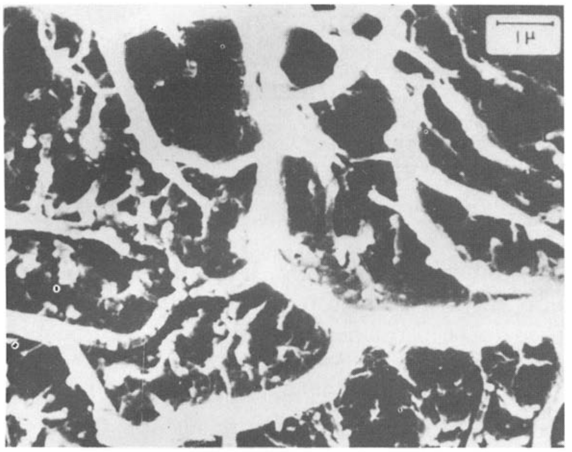


Fig. 11. Scanning electron micrograph of partially dried agarose–polyacrylamide composite supports (from ref. 92).

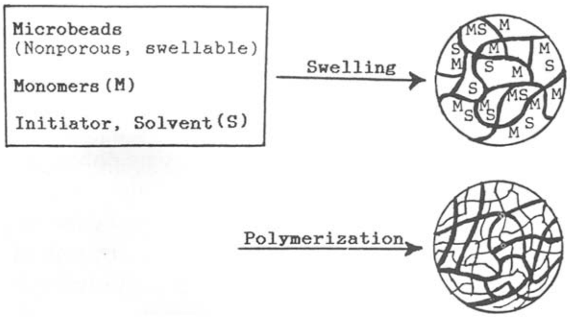


Fig. 12. Preparation of beaded interpenetrating networks.

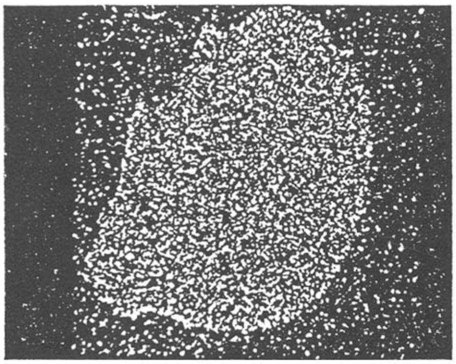


Fig. 13. X-ray microprobe image of a cross-linked (network) copolymer of dimethylacrylamide and vinylferrocene formed within a preformed network of cross-linked polydimethylacrylamide [94].

shows that the organometallic network is homogeneously distributed throughout the initial polyacrylamide network.

6.3. Core–shell grafts

An ideal model of a core shell graft composite, having a rigid core and relatively long flexible graft chains, is shown in Fig. 14A. The rigid core may be an inorganic material (*e.g.* glass) or an organic polymer (*e.g.* polystyrene). Such an ideally grafted surface can be produced by attaching specifically monofunctionalized linear chains to suitably activated surfaces. A more practical approach is, however, the attachment of initiator groups [95] or vinyl residues [96] to the surface, followed by direct graft polymerization. Graft polymerization onto the surface of the core produces a different shell structure (Fig. 14B) as a result of chain transfer and termination reactions. Note that when the core particles are porous, the illustrations in Fig. 14 still apply, albeit the overall morphology approaches that of pore-matrix composites shown in Fig. 10. For representative examples of these preparations, see refs. 97–100 and section 5.4 in Part II.

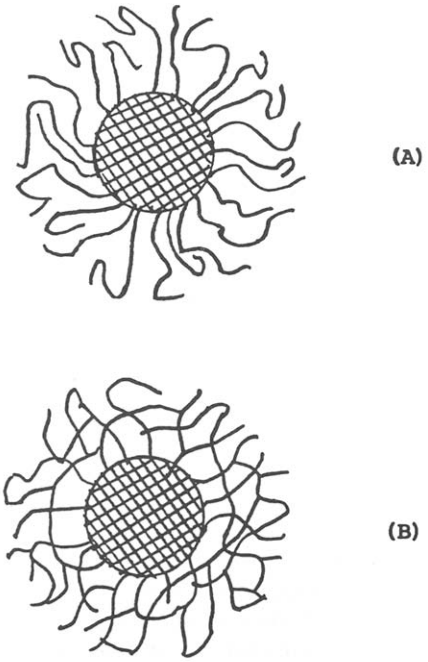


Fig. 14. Schematic presentation of core–shell grafts. (A) Ideal structure; (B) typical structure available via attachment of monomer on the surface, followed by graft polymerization.

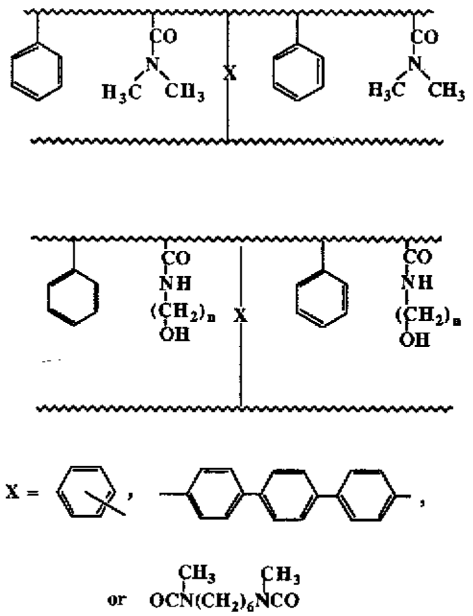


Fig. 15. Amphiphilic polymer supports with copoly(styrene–acrylamide) structures.

6.4. *Pellicular supports*

Pellicular supports are essentially similar to core–shell grafts shown in Fig. 14B. However, the term "pellicular" is used to describe materials in which the outer shell is relatively thicker than that in core–shell supports. In addition, in pellicular supports the shell is deliberately cross-linked by the inclusion of a cross-linking monomer, and the shell may or may not be covalently attached to the core. Here again, the pellicular terminology is equally used whether the core is rigid or porous. The topic of pellicular supports has been reviewed thoroughly by Horváth [101].

7. COPOLYMER SUPPORTS

A logical extension of the concept of composite supports is the development of copolymer supports. In a formal sense, all of the cross-linked beaded resins and most of the composite supports described above are copolymers. In addition, activated (functionalized) polymer supports (see Part II) may also be regarded as copolymers. However, in all of these, the main repeating units on the polymer *backbone* have essentially the same structure. The term "copolymer support" is here meant to describe a copolymer structure incorporating two (or more) distinctly different types of monomeric units in the polymer *backbone*.

An interesting class of copolymer supports is the alternating copoly(styrene–acrylamide) structures shown in Fig. 15. These copolymers combine the structural units of polystyrene with those of polydimethylacrylamide or polyhydroxyalkylacrylamide. As a result, they cover the solvent and substrate compatibility ranges of both hydrophobic and hydrophilic polymer supports, and hence are suitable for general use in aqueous media and in polar and non-polar organic solvents. The new copolymer supports are also ideally suitable for multi-step processes involving the use of aqueous and non-aqueous solvents at different stages.

It is interesting that beaded copolymer supports of the type shown in Fig. 15 cannot be produced by suspension copolymerization of the respective comonomers. This is because the two comonomers have opposite water solubilities and unfavourable copolymerization reactivity ratios. These copolymer supports are, however, obtained readily by a new synthetic method based on the chemistry of "activated esters" or "leaving group substitution" [102, 103].

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