Journal of Chromatography, 586 (1991) 199–219
Elsevier Science Publishers B.V., Amsterdam

CHROM. 23 549

Review

Beaded polymer supports and gels

II. Physico-chemical criteria and functionalization

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(First received March 13th, 1991; revised manuscript received June 11th, 1991)

ABSTRACT

A systematic description of morphology, physicochemical criteria, functionalization and activation of beaded polymer supports and gels is presented. The products covered include polystyrene, polyacrylamides, copoly(styrene-acrylamide)s, polysaccharides, polymethacrylates and silica gel. Morphological aspects of beaded polymer products (i.e. bead size, porosity and surface area) and swelling behaviour are discussed. Various chemical reactions employed for derivatization and activation of polymer supports and gels are charted and their limitations and side reactions are outlined. The significance of physicochemical criteria such as matrix architecture, chemical structure of the polymer backbone, site accessibility and spacer arm are also briefly covered.

CONTENTS

1. INTRODUCTION

Microspherical polymer products (beaded polymer supports and gels, both organic and inorganic) are widely used as packing materials for chromatography and a variety of other applications (see Table 1) [1–20]. Gel (permeation) chromatography (or gel filtration) [1–3] is based on the pore structure of the

polymer microbeads. Porosity and surface area also play an important role in other applications such as ion-exchange and affinity chromatography and polymer-supported catalysis. However, in these and most other uses of polymer supports listed in Table 1, the "function" of the polymer is based essentially on specific functional residues (or reactive sites). In either case, the size of the microbeads, the swelling behaviour and the chemical structure of the polymer backbone strongly influence the overall performance of the product.

This review follows the general introduction and preparative details of beaded polymer products discussed in Part I [21]. The present discussion focuses on morphological, chemical and physicochemical aspects of beaded polymer supports and gels, including polystyrene, polyacrylamides, polymethacrylates, polysaccharides, silica gel and copoly(styrene–acrylamide)s. The manufacturing basis of bead size, porosity, surface area and bulk expanded volume are discussed. Various chemical reactions employed for the functionalization and activation of beaded polymer supports and gels are systematically reviewed. The significance of physico-chemical criteria, such as the chemical structure of the polymer backbone, architecture of the polymer matrix, site accessibility and spacer arm are also pointed out.

2. PARTICLE SIZE

Beaded polymer supports and gels are produced by two-phase suspension processes in which "microdroplets" of a monomer or polymer solution are directly converted to the corresponding "microbeads" (see Part I). The size of the microdroplets (and hence that of the microbeads) is determined by a number of interrelated manufacturing parameters, including reactor design, the rate of mixing (stirring), ratio of the monomer (or polymer) phase to the suspension medium, viscosity of both phases and type and concentration of the droplet stabilizer [22–24].

The size distribution of the polymer beads obtained by two-phase suspension systems depends mainly on the configuration of the reactor and "artful" management of the suspension process. With the cylindrical apparatus introduced in Part I, it is possible to obtain relatively uniform beads in which the deviation from the average size is not greater than about 100% (see Figs. 3 and 4 in Part I) [22]. More generally, however, two-phase suspension systems produce beaded products with considerably broader particle size distributions (e.g., 5–50 or 20–200 μm). Such products are usually separated (graded) into a series of relatively narrow particle size ranges as desired. The actual classifica-

TABLE 1

MAJOR APPLICATIONS OF BEADED POLYMER SUPPORTS AND GELS

Application	Bead functionality needed or preferred[a]	Ref.
Chromatography:		
Gel (permeation) or size exclusion	Porosity	1–3
Ion-exchange	SO_3H (Na), CO_2H (Na), NR_3X	4–6
Affinity	OH, NH_2, CHO COOH, COOAr	7–9
Enantioselective	Asymmetric centers (*e.g.*, *C)	10
Biotransformations (immobilized enzymes/cells)	OH, NH_2, COOH, porosity	11, 12
Solid-phase peptide synthesis	OH, NH_2	13, 14
General organic synthesis	Various	15, 16
Chemical catalysis	PPh_3, NC, CN, others	15–17
Hydrometallurgy (metal ion extraction)	Various	18
Diagnostics and immunoassay	OH, NH_2, CHO, COOH	19, 20

[a] R = alkyl; X = Br, Cl, OH; Ar = activating/leaving group; Ph = phenyl.

tion process depends on the size range involved, the nature of the beaded product and its intended application. Relatively large (>50 μm) and mechanically stable particles can be easily sieved in the dry state. Smaller particles are processed more conveniently in the swollen (or wet) state. Highly porous particles may be fragile and irregular in the dry state. For these, and also for very fine particles (<20 μm), classification is accomplished by wet sedimentation, counterflow settling (elutriation) or counterflow centrifugation [25–27] (see also ref. 1, pp. 109–112). Fig. 1 shows the particle size distribution of a typical polystyrene resin and those of its fractions obtained by the counterflow centrifugation method.

Among various factors influencing particle size, stirring speed (or more generally, the power of mixing) provides a relatively convenient means of particle size control for most practical purposes. Fig. 2 illustrates a typical example [28] of the effect of stirrer speed on the size of polystyrene particles obtained by suspension polymerization. The pattern of particle size variation *versus* stirrer speed indicated in Fig. 2 applies equally to beaded polymers obtained by other two-phase suspension systems discussed in Part I.

It must be emphasized, however, that there are limits within which particle size can be controlled by the adjustment of the stirring speed. These limits

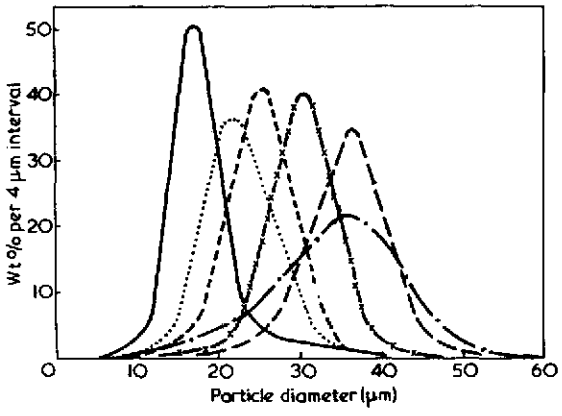


Fig. 1. Particle size distribution of a typical polystyrene–DVB resin produced by suspension polymerization (— · —), and its fractionation by an Alpine Zig-Zag Centrifugal Separator. Particle size: (———) 10–15 μm; (.....) 16–20 μm; (— — —) 20–24 μm; (— × —) 25–28 μm; (— — —) >28 μm (adapted from ref. 27).

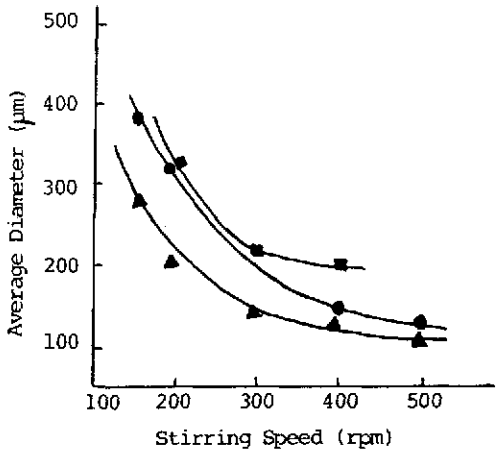


Fig. 2. Effect of stirring speed on the size of polystyrene particles produced by suspension polymerization; stabilizer: (■) 0.2%; (●) 0.3%; (▲) 0.4% (adapted from ref. 28).

depend on the size and the configuration of the polymerization reactor (including its stirring arrangement). For laboratory preparations involving a total volume of about 500 ml (see Fig. 3 in Part I), the stirring speed can be varied between about 200 and 800 rpm. Lower stirring speeds may not be sufficient to establish a steady-state droplet size distribution, whereas too vigorous stirring may exceed the shear tolerance of the whole set-up.

Another practically important consideration about the dependence of particle size on stirring speed is that smaller droplets/particles produced by faster mixing require correspondingly increased concentrations of the droplet stabilizer. In the absence of sufficient stabilizer, the smaller droplets coalesce easily during the hardening stage. This produces larger (and irregularly sized) particles, and may also lead to partial or full coagulation of the microbeads. For example, in the case of experiments indicated in Fig. 2, at a stabilizer concentration of 0.1%, an increased rate of stirring leads to the formation of much larger particles.

In principle, two-phase suspension systems can be employed to produce polymer particles within the range of about 0.2–2000 μm. In suspension polymerization of vinyl monomers, however, the production of very small particles (<20 μm) is difficult owing to emulsification and latex formation by emulsion polymerization. This problem does not arise when the particles are formed by solvent

TABLE 2

DEPENDENCE OF SURFACE AREA AND POROSITY OF SILICA GEL ON THE CONDITIONS OF HYDROTHERMAL TREATMENT (ADAPTED FROM REF. 32)

Sample	Treatment conditions			Surface area and porosity		
	Temperature (°C)	Time (h)	Pressure (bar)	Surface area (m^2/g)	Pore volume (ml/g)	Mean pore diameter (nn)
1a	...	–	–	210	0.73	10.0
1b	110	4	2	121	0.70	22.0
1c	180	4	10	39	0.72	74.0
1d	250	4	50	20	0.78	290
1e	300	4	100	1.4	0.70	1420
2a	–	–	–	330	1.07	10.5
2b	250	5	50	63	1.09	68.0
2c	250	10	50	51	1.06	88.5
2d	250	15	50	48	1.15	88.0
2e	250	20	50	38	1.06	88.5
3a	–	–	–	498	0.63	5.1
3b	100	0.5	1	432	0.93	7.2
3c	100	1.0	1	395	0.94	8.0
3d	100	1.5	1	356	0.94	8.6

extraction and suspension cross-linking (*e.g.* polysaccharide gels). Small particles of vinyl-based polymers can be obtained by dispersion polymerization or by more elaborate two-step processes involving the enlargement of monodisperse seed particles [29].

3. POROSITY AND SURFACE AREA

Traditional sorbents such as charcoal [30,31] and silica gel [32] have rigid three-dimensional structures with tightly fixed matrices. Accordingly, surface area and porosity in inorganic supports represent real structural criteria, and often the limits of support characterization. In contrast, organic gels are based on relatively flexible matrix structures (see Fig. 2 in Part I). Here, porosity and surface area represent tertiary and higher orders of macromolecular structure, rather than the limits of structural characterization.

Porosity and surface area in both inorganic and organic supports can be controlled easily during production. In silica gel, the pore structure is dependent on hydrothermal treatment (Table 2) [32,33] and on other manufacturing parameters discussed in Part I. In the case of organic resins, porosity is determined by gelation and/or precipitation processes that take place during the conversion of liquid microdroplets to solid microbeads. For example, polystyrene beads produced in the presence of 1 2% divinylbenzene (DVB) without a monomer diluent have very low surface area (< 1 m^2/g) with no real porosity or very small pores. However, by using higher DVB concentrations and a monomer diluent, polymer beads with a wide range of porosities can be produced, depending on the proportions of DVB and monomer diluent.

Fig. 3 shows scanning electron micrographs of two samples of beaded copolymers of styrene with 2,4,5-trichlorophenyl acrylate and DVB, obtained in the presence of either chlorobenzene or chlorobenzene–octane [34]. In the presence of chlorobenzene (a good solvent for this polymer), the polymer chains remain solvated throughout the matrix formation. This produces a relatively homogeneous matrix with very low porosity (micrograph a). On the other hand, polymerization in the presence of chlorobenzene–octane (a poor solvent) leads to phase separation and the formation of polymer "nuclei" within the polymerizing droplets. Accordingly, each individual polymer bead produced in this

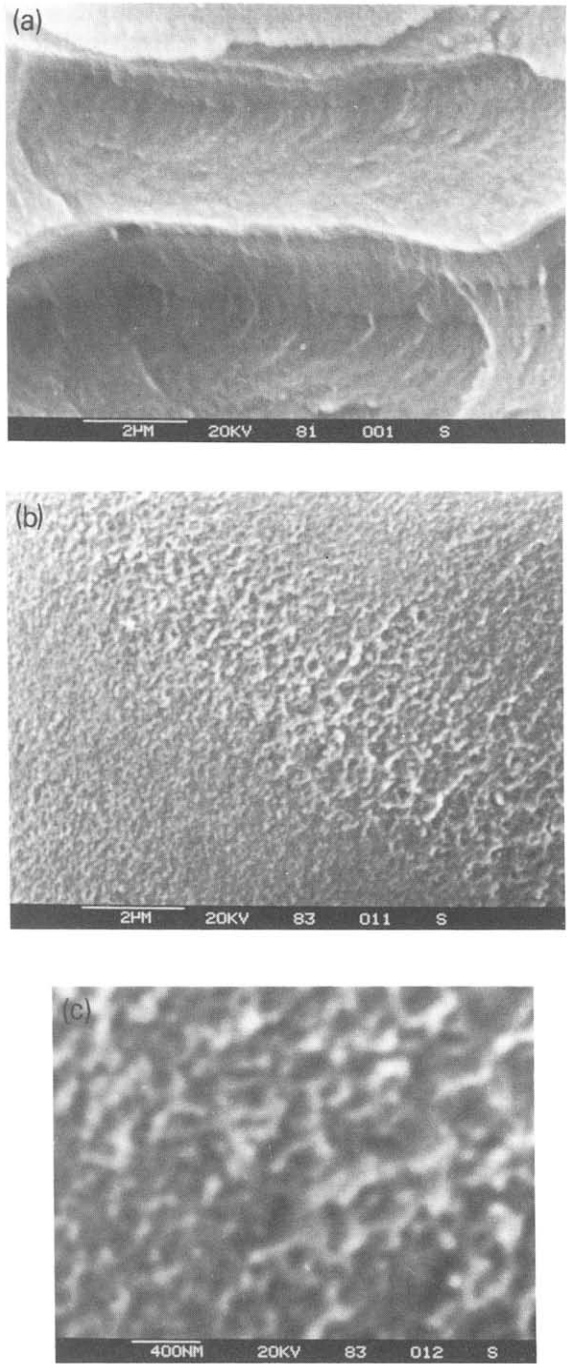


Fig. 3. Scanning electron micrographs of cross-sections of beaded copolymers of styrene with 2,4,5-trichlorophenyl acrylate and DVB obtained in the presence of chlorobenzene (a) (a good solvent) or (b and c) chlorobenzene–*n*-octane (a poor solvent); (c) is the same as (b) but with higher magnification (from ref. 34).

way consists of a mass of aggregated polymer nodules or "grains" evident at a magnification of 10 000 (micrograph b). At a higher magnification of 40 000 (micrograph c), the inter-grain spaces (*i.e.* pores) with dimensions of about 20–200 nm are also clearly visible. It must be emphasized again, however, that porosity in organic polymer supports may not represent a strictly invariable criterion [35].

Control of porosity by means of a monomer diluent (or porogen) has been extensively studied for polystyrene [35–38] and polymethacrylates [39–42]. For polyacrylamides, a detailed electron microscopic study of gels produced in bulk was reported by Ruchel and Brager [43]. A recent illustration of the dependence of pore size distribution on the nature and percentage of monomer diluent is provided in Fig. 4 [44]. The surface areas of the resins indicated in Fig. 4 range between 5 and 145 m^2/g.

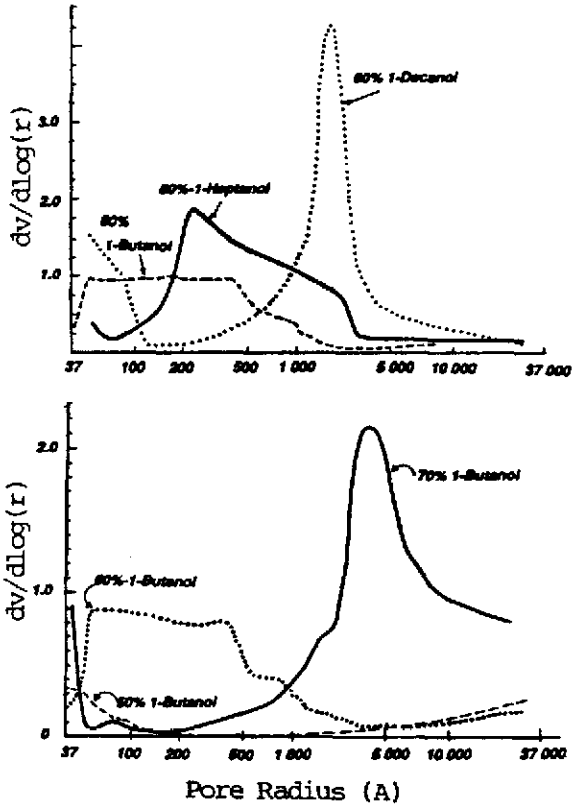


Fig. 4. Dependence of porosity of phenolic resins on the nature and proportion of monomer diluent. r = Pore radius; v = pore volume (adapted from ref. 44).

Surface area and porosity are routinely measured by nitrogen adsorption–desorption (BET and BJH methods), mercury intrusion and low-angle X-ray scattering [45–48]. Specific pore volume can be estimated from the apparent density of the microbeads or the gain of an inert liquid (a non-solvent for the polymer). These methods are generally convenient and suitable for comparison of samples produced under related experimental conditions. However, the absolute values of the data obtained by these methods are subjective to some extent. Electron microscopy (EM) provides directly visual evidence of pore size and pore size distribution (see Fig. 3), but it is less practicable for routine use. Thus, a combination of EM and conventional methods of pore size measurement should provide reliable information on the pore structure of the polymer.

Matrix porosity is the basis of support characteristics in gel (permeation) chromatography, and determines the fractionation range of the support. Resin porosity may also affect the support performance in other applications such as affinity chromatography, catalysis and solid-phase synthesis. However, in all of the above applications, the support functions in a solvent in which the matrix may swell to various extents. Under these conditions, the specific pore volume and pore size distribution in the swollen state [49] may be substantially different from those measured in the dry state. Accordingly, the support performance is strongly dependent on its swellability in the solvent used.

4. POLYMER SWELLABILITY

Resin swellability (or bulk expanded volume) in a given solvent is a multifaceted property reflecting the chemical structure of the polymer backbone, degree of cross-linking and the architecture of the polymer matrix. The three-dimensional structure of the polymer matrix (or network) *takes shape* according to the conditions prevailing during the formation of the polymer microbeads. For beaded poly-

TABLE 3

EFFECTS OF CROSS-LINKING AND MONOMER DILUENT ON THE SWELLING BEHAVIOUR OF STYRENE-BASED RESINS[a]

Cross-linking (mol%)	Monomer diluent (ml/g)[b]	Bulk expanded volume (ml/g) in solvent[c]					
		None	MET	DMF	EAC	DOX	DCM
0.5	0.0	nm[d]	2.5	6.2	6.5	10	11
1.4	0.0	1.6	2.2	4.2	4.8	5.0	5.2
2.2	0.0	1.4	2.0	2.3	2.6	3.6	3.5
5.0	0.0	1.4	nm	1.8	nm	nm	2.1
10	0.0	1.4	nm	1.7	nm	nm	1.9
2.1	A(1.0)	1.6	2.4	5.4	6.1	9.6	10
2.1	B(1.0)	6	6	10	20	20	22
2.1	C(1.0)	4.4	5.1	6.8	7.0	8.5	8.5
2.1	D(1.0)	1.6	1.6	4.2	4.5	5.7	6.2
5.8	A(3.3)	1.6	1.8	6.1	8.5	9.5	11
5.8	B(3.3)	14	16	17	15	14	25
5.8	C(3.3)	7	nm	nm	nm	nm	11
5.8	E(2.5)	2.1	3.4	5.5	5.5	7.0	8.5
5.8	F(3.0)	6	nm	nm	nm	nm	11
5.8	A(5.0)	nm	Soluble	Soluble	Soluble	Soluble	Soluble

[a] Produced [50] by suspension copolymerization of styrene and approximately equal concentrations of 2(3)-ethylvinylbenzene and 2(3)-divinylbenzene.

[b] Monomer diluent (ml/g monomer); A = chlorobenzene; B = dodecane; C = pentanol; D = carbon tetrachloride; E = A + B (2:3); F = B + C (2:1).

[c] MET = Methanol; DMF = dimethylformamide; EAC = ethyl acetate; DOX = dioxane; DCM = dichloromethane.

[d] nm = Not measured.

mer supports obtained by suspension polymerization, the type and percentage of the monomer diluent strongly influence the shape of the three-dimensional polymer network. For polysaccharide gels produced by suspension cross-linking, the nature and proportion of the polymer solvent have a similar role. In either case, polymer molecular weights (degree of polymerization, DP) also contribute to matrix architecture. In general terms, these criteria also apply to inorganic gels, although the process is more complicated in this case (see Part I).

Table 3 [50] shows the swelling behaviour of a series of copoly(styrene–divinylbenzene) resins in a number of commonly used organic solvents, including methanol (MET), dimethylformamide (DMF), dioxane (DOX), ethyl acetate (EAC) and dichloromethane (DCM).

A full interpretation of the swelling data in Table 3 is beyond the scope of the present discussion. However, a number of general conclusions can be drawn from the swelling patterns of different samples. For example, under a given set of experimental conditions, the extent of polymer swelling (bulk expanded volume) decreases as the nominal degree of cross-linking increases. It is also evident that, for a given degree of cross-linking, the bulk expanded volume is strongly dependent on the nature and proportion of the monomer diluent used during matrix formation. In general, the higher the percentage of the monomer diluent, the larger the bulk expanded volume of the resin, but different diluents affect polymer swelling to different extents.

An interesting implication of these observations is that the bulk expanded volume of the gel can be maintained at a relatively constant level by simultaneously increasing both the degree of polymer cross-linking and the percentage of the monomer diluent. However, this process has a far-reaching effect on the gelation and precipitation of the polymer "grains" within the microbeads (see Fig. 3). The pattern of gelation and precipitation, in turn, affects the porosity and surface area of the beads, as discussed in the preceding section.

Another practically important aspect of resin swellability is the pattern of polymer–solvent compatibility, *i.e.* the relative measure of polymer swelling in different solvents. Polymer–solvent compatibility is determined by the chemical structure of the polymer backbone. An interesting illustration of this structure–property relationship is provided by the swellability data in Table 4 [32]. These data show the swelling behaviour of a series of copoly(styrene–

TABLE 4

SWELLING BEHAVIOUR OF DIFFERENT TYPES OF POLYMER SUPPORTS

Polymer type[a]	Swellability (ml/g) in different solvents[b]								
	TOL	EAC	THF	DCM	DMF	DMSO	MeOH	AcOH	Water
Polystyrene:									
(1a)	5.1	4.8	5.0	5.2	4.2	–[c]	–	–	–
(1b)	10.0	8.5	10.0	11.0	6.2	–	–	–	–
Polydimethylacrylamide:									
(2a)	–	–	–	9.5	9.1	10	12	12	9.0
(2b)	–	–	–	20	20	20	23	35	19
Copoly(styrene–dimethylacrylamide)									
(12b)	7.1	6.0	7.5	7.3	6.0	5.1	6.1	6.9	3.9
(12c)	4.7	4.0	5.3	5.8	5.2	4.6	5.5	5.5	3.7
(12d)	18	16	21	27	16	13	13	21	8.9

[a] For details of polymer types 1 and 2, see refs. 51 and 52, respectively. Polymers 12b, 12c and 12d were obtained from three different samples of copoly(styrene–2,4,5-trichlorophenyl acrylate) according to Fig. 17.

[b] TOL = Toluene; EAC = ethyl acetate; THF = tetrahydrofuran; DCM = dichloromethane; DMF = dimethylformamide; DMSO = dimethyl sulphoxide; MeOH = methanol; AcOH = acetic acid.

[c] Dashes indicate polymer–solvent incompatibility.

dimethylacrylamide)s as compared with those of the corresponding homopolymers, polystyrene and polydimethylacrylamide.

The hydrophobic resin (polystyrene) [51] and the hydrophilic resin (polydimethylacrylamide) [52] are compatible with, respectively, the first five and the last six solvents listed in Table 4. The copolymer resins, copoly(styrene–dimethylacrylamide)s, incorporate the structural units of both homopolymers, and have an amphiphilic structure. As a result, they are compatible with all of the solvents listed in Table 4, ranging from toluene and ethyl acetate on the one hand to dimethyl sulphoxide and water on the other. This general solvent compatibility is related to the actual solvation of the polymer backbone, and should not be confused with the uptake of non-solvents by porous gels (see below). Swellability data for other polymers covered by this review have been reported by Pharmacia [53] for Sephacryl, Peska *et al.* [54] for cellulose ion exchangers, Smrz and Viska [55] for Spheron, Fenyvesi *et al.* [56] for polycyclodextrins, Epton and co-workers [57,58] for Enzacryl and related polymers and Birr [59] for low-cross-linked polystyrene.

It should be emphasized that the extent of apparent polymer swelling (bulk expanded volume) and the solvation of the polymer chains do not necessarily coincide. The distinction between "polymer swelling" and "solvation of the polymer chains" is particularly relevant in any discussion of the reactivity (or site accessibility) of polymer-bound reactive sites. When a low-cross-linked polymer (whether porous or not) swells in a "good solvent", individual polymer chain segments become solvated. Under these conditions, the polymer-bound reactive sites are rendered potentially accessible to the soluble reagent. On the other hand, highly cross-linked porous gels generally "suck up" certain volumes of various liquids, whether good or poor solvents [60,61]. Here, the liquid is stored in the pores (*cf.* the channels between the precipitated grains in Fig. 3), without necessarily contributing to polymer solvation and site accessibility. For non-porous polymer beads, the extent of swelling is closely related to solubility parameters [62], that is, the closer the solubility parameters of the polymer and the solvent, the greater the extent of polymer swelling.

In practice, highly swollen gels may not be desirable, because they collapse under pressure and are difficult to filter. In addition, a highly swollen gel behaves as a viscous polymer solution, with the consequence of poor substrate diffusion and transport within the polymer matrix. Accordingly, it is often necessary to employ relatively more cross-linked porous gels, in which site accessibility is judiciously compromised at the expense of handling convenience and rapid diffusion. This topic is further discussed in section 6.1.

5. ACTIVATION AND FUNCTIONALIZATION

The discussion on the activation and functionalization of polymer supports and gels is organized under separate subheadings for major polymer types, including polystyrene, polyacrylamides, polysaccharides, porous silica and the recently introduced amphiphilic copolymers. Other polymer types commonly used for chromatography and related applications include poly(vinyl alcohol), poly(hydroxyethyl methacrylate) (Separon), poly(glycidyl methacrylate) (Eupergit) and Ultragel. The first two resins contain hydroxy groups, and can be activated and derivatized in basically the same way as described for polysaccharides. Polymer supports carrying glycidyl (oxirane or epoxide) functionality react with nucleophiles in a manner similar to oxirane derivatives of polysaccharides and silica gel. Ultragel contains both hydroxy and amide residues, and can be derivatized by the same procedures as described for polysaccharides and polyamide. Activation of organic and inorganic supports by complexation/chelation of titanium and related metals (for enzyme immobilization) has been reviewed recently [63], and will not be covered here. For derivatization of fluorocarbon polymers (Kel-F beads) by organometallic reagents, see refs. 64 and 65.

5.1. Polystyrene

Beaded copolymers of styrene and divinylbenzene are most widely used for the manufacture of strongly acidic [66] and strongly basic [67] ion-exchange resins [68,69]. Commercially important polystyrene ion exchangers are produced in one or two steps, as depicted in Fig. 5. A variety of related chelating agents [18] can also be produced from the chloromethylated polystyrene by processes basically similar to that of the ammonium resins shown in Figure 5.

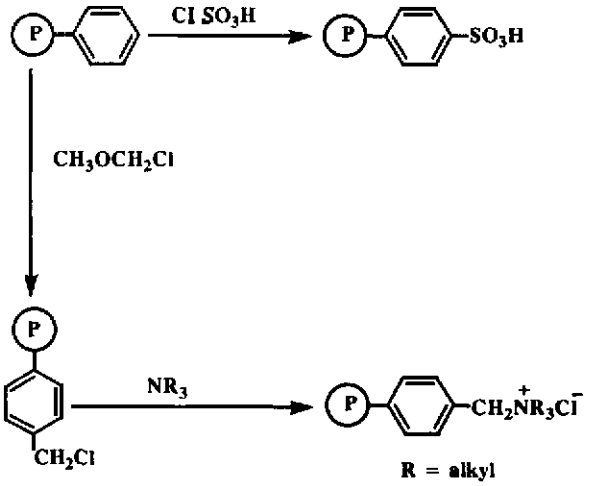


Fig. 5. Basic chemical reactions employed for the synthesis of styrene-based ion exchangers.

On the basis of its chemical structure, polystyrene is more inert than other commonly available polymer supports. Largely for this reason, and also owing to compatibility with organic solvents, styrene-based polymer supports have been generally adapted for solid-phase peptide synthesis [13,14]. They are also being studied for a wide range of other analytical, catalytic and synthetic uses [15–17].Chloromethylation (see Fig. 5) [66–72] and bromination [73,74] of beaded polystyrene provide two of the most useful intermediates for the synthesis of styrene-based polymer supports [15–17]. It must be borne in mind, however, that both chloromethylation and bromination involve a variety of side-reactions and complications [75], depending on the experimental conditions and the desired level of functionalization.

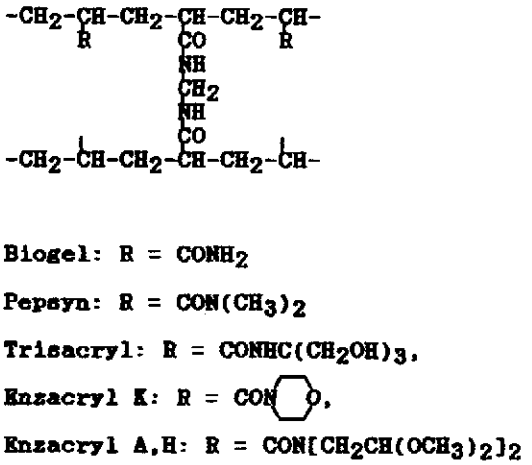


Fig. 6. Structures of different acrylamide gels.

5.2. *Polyacrylamides*

There are five different types of acrylamide-based polymer supports and gels commercially available, including polyacrylamide (Bio-Gel), polyacryloylaminomethyldimethylacetal (Enzacryl A and H), polyacryloylmorpholine (Enzacryl K), poly[(trishydroxymethyl)methylacrylamide] (Trisacryl) and polydimethylacrylamide (Pepsyn). The structures of different polyacrylamides are shown in Fig. 6.

Derivatization of Bio-Gel, as elaborated by Inman and Dintzis [76], is outlined in Fig. 7. The functionalized gels obtained in this way can be further derivatized and activated (*i.e.* for enzyme and ligand attachment) by reagents such as nitrous acid, carbodiimide and glutaraldehyde [76]. Isocyano derivatives of Bio-Gel have also been produced by Goldstein [77] for enzyme immobilization via four-component condensation (4CC). For a review of these and other isocyano polymer supports, see ref. 78.

Trisacryl gels contain an abundance of hydroxy groups and can be activated in basically the same way as described for polysaccharide gels (see below). Enzacryl A and H are produced by reaction of the dimethylacetyl resin with tartaric acid dihydrazide

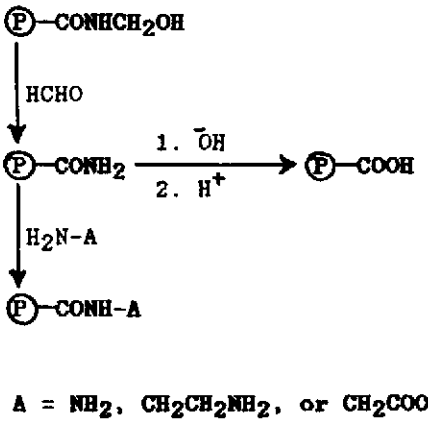


Fig. 7. Functionalization of polyacrylamide (Bio-Gel).

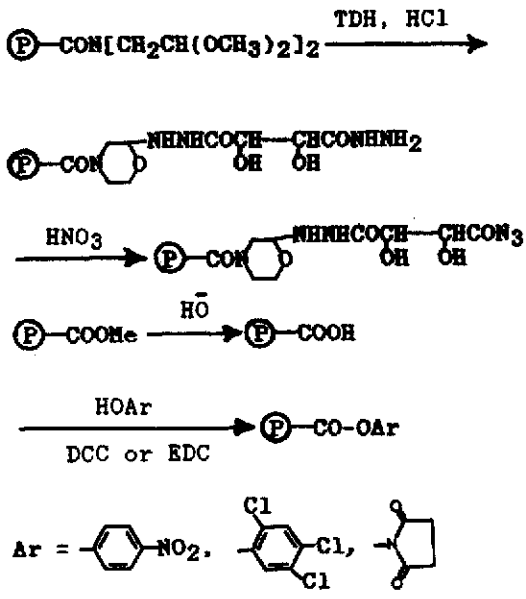


Fig. 8. Preparation of activated derivatives of substituted acrylamide gels. Enzacryl (adapted from ref. 79) and Pepsyn (adapted from ref. 52). TDH = Tartaric acid dihydrazide; DCC = dicyclohexylcarbodiimide; EDC = 3-(dimethylaminopropyl)ethylcarbodiimide; Me = methyl.

and nitrous acid, as depicted in Fig. 8 [79]. Dimethylacrylamide resins have been developed for solid-phase peptide synthesis [80] (hence the acronym Pepsyn). Details of the synthesis and derivatization of these resins, including those carrying free amino or carboxy functionality, have been discussed recently [52]. Preparation of carboxyl-activated dimethylacrylamide resins is also outlined in Fig. 8 [52].

Enzacryl K was introduced by Epton for gel permeation chromatography (ref. 2, pp. 70–90). Derivatization of this polymer via treatment with diamines at relatively high temperatures has been reported by Narang *et al.* [81] and Arshady *et al.* [82,83]. Their work represents an interesting functionalization route in which the reactive sites on the polymer are the "cross-linking units", as indicated in Fig. 9 [82,83]. Accordingly, the result of the functionalization depends strongly on the structure of the diamine and the reaction conditions employed (usually DMF or ethylene glycol solvent, 150–200°C, 2–24 h).

With symmetrical diamines, initially derivatized gels with free amino groups are produced, followed by the gradual formation of highly rigid resins if the reaction is continued. When unsymmetrical diamines, such as 1-(2-aminoethyl)piperazine, are used, gradual de-cross-linking of the gel leads to the formation of completely soluble polymers.

5.3. Polysaccharides

Polysaccharide gels are produced from cellulose, agarose (Sepharose) and dextran (Sephadex), and they are widely used for chromatography and enzyme immobilization. All of these polymers contain hydroxy groups available for activation and further derivatization. A small number of hydroxy groups in native agarose are sulphated ($-CH_2OSO_3^-$), but these sulphate groups are usually removed during the manufacture of the beaded polymer. Dextran-based gels may contain some carboxyl groups. It is also noteworthy that the basic polysaccharide structures of these gels is preserved only in the case of non-cross-linked products. In cross-linked polysaccharide gels, the chemical structure is often substantially altered, depending on the nature and extent of the cross-linking units. Two examples, namely those of agarose gels produced by epichlorohydrin cross-linking and Sephacryl obtained by cross-linking (copolymerization) of allyldextran with bisacrylamide, are shown in Fig. 10.

It is also evident from Fig. 10 that cross-linked polysaccharide gels may carry primary and/or secondary hydroxy groups. The proportions of different OH groups on the polymer are determined by the type of polysaccharide, and the structure and the percentage of the cross-linking units. This must be borne in mind when planning the activation and utilization of polysaccharide gels. Primary hydroxy groups are, for the purpose of the present discussion, substantially more reactive than secondary and tertiary ones. This order of reactivity also applies to the corresponding activated derivatives such as sulphonates (see below).

Chemically modified polysaccharide gels can be divided into two broad categories, namely ion-exchange resins and activated intermediates used for affinity chromatography and enzyme immobilization. Preparation of various cellulosic ion exchangers was described by Peterson and Sober in the late 1950s [84,85]. Determan and Wieland in the 1960s

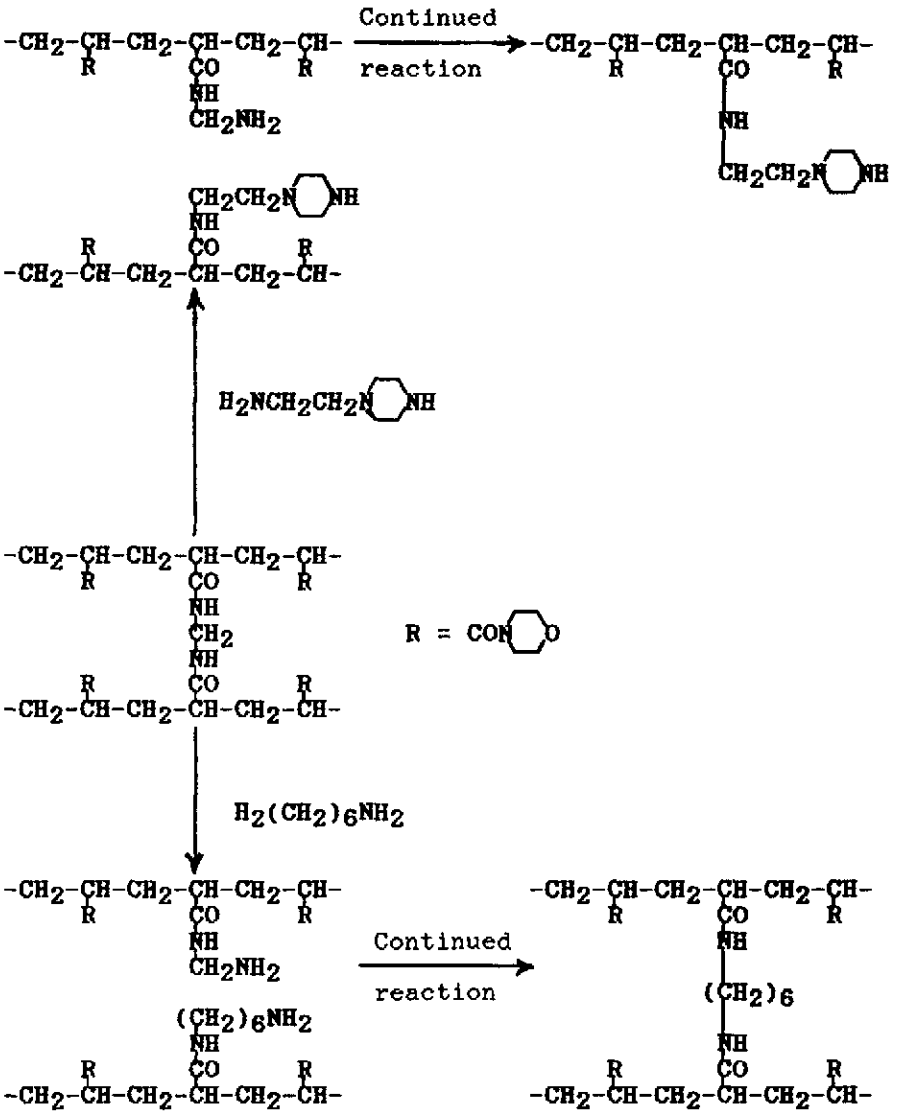


Fig. 9. Functionalization of Enzacryl K via treatment with diamines [82].

[86], and more recently by Peska *et al.* [54]. The basic chemistry of these preparations is illustrated in Fig. 11.

The use of polysaccharide-based polymer supports for affinity chromatography and enzyme immobilization involves the preparation of "activated" gels, followed by the attachment of the affinity ligand or enzyme via NH_2, SH and/or OH groups. One generally useful type of activated intermediate for this purpose is the "active ester derivative" obtained by succinylation, followed by reaction with a phenolic or N-hydroxy compound, as indicated in Fig. 12. The succinylation reaction is carried out in basically the same way as the reactions shown in Fig. 11. A wide range of other commonly used activation methods are outlined in Fig. 13 [87–101]. It should be evident that most of the chemical reactions indicated in Figs. 12 and 13 are

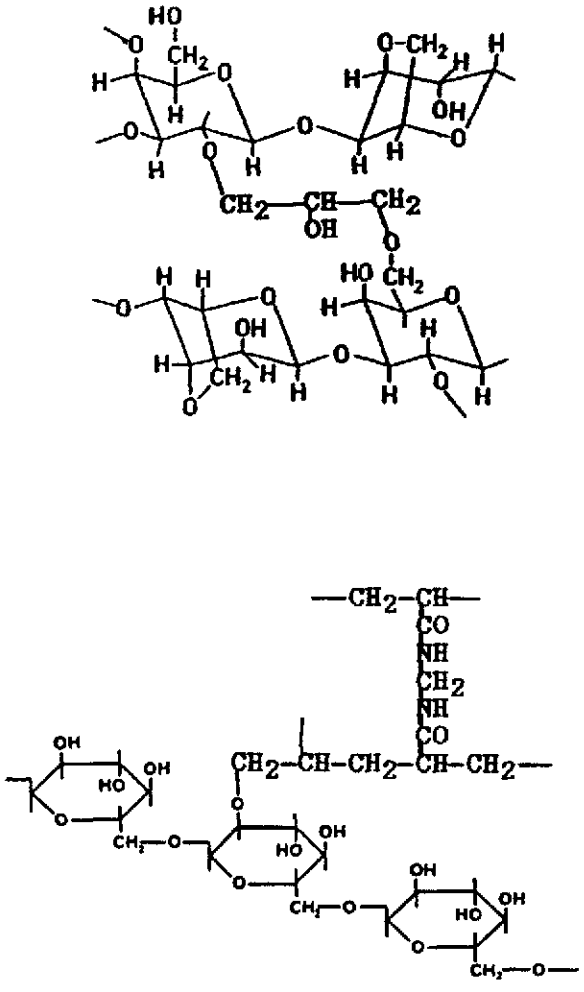


Fig. 10. Structures of Sepharose (cross-linked agarose) (top) and Sephacryl (copolymer of allyldextran and bisacrylamide) (bottom).

also applicable to other hydroxyl-bearing polymers such as Trisacryl, Separon, poly(vinyl alcohol) and hydroxy derivatives of silica gel and glass beads.

Among the reactions represented in Fig. 13, cyanation with cyanogen bromide was first introduced by Axen *et al.* [87] in 1967 and is still widely used. The popularity of this activation route is largely due to the simplicity of the method and the low cost of the reagent. However, the procedure usually requires a large excess of the toxic reagent (cyanogen bromide). Furthermore, the linkage formed between the cyanyl-activated gel and the

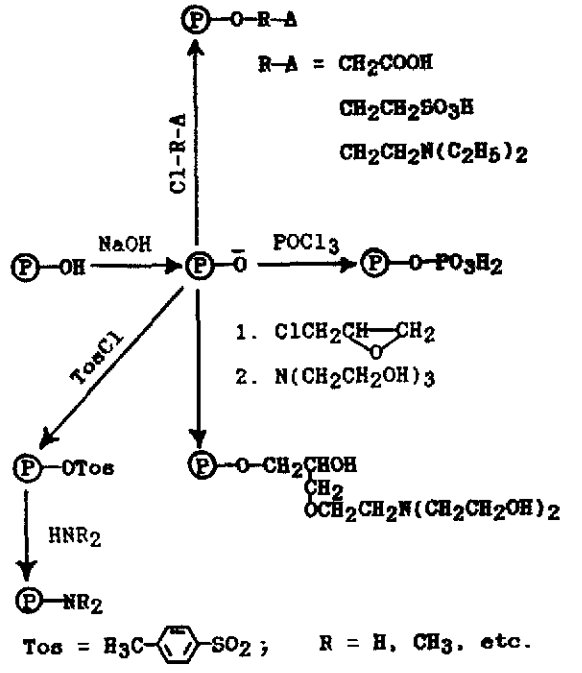


Fig. 11. Preparation of cellulose-based ion exchangers.

ligand (or enzyme) is not completely stable, and hence gradual leakage and decreased capacity (or activity) of the gel occur.

Wilchek [91] studied the mechanistic details of cyanogen bromide activation. He introduced less toxic cyanylating reagents, such as triethylamine cyanogen bromide complex and 4-nitrophenyl cyanate. However, the reduction in toxicity achieved by

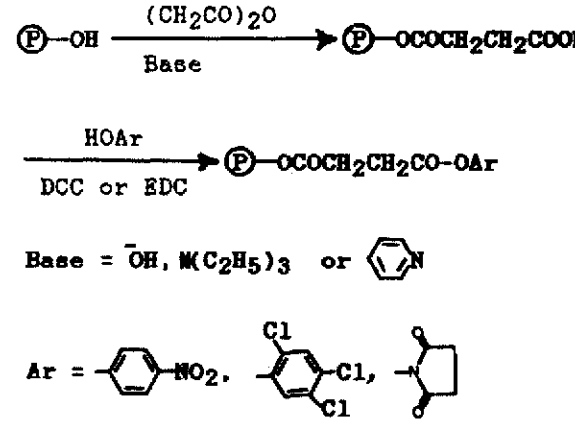
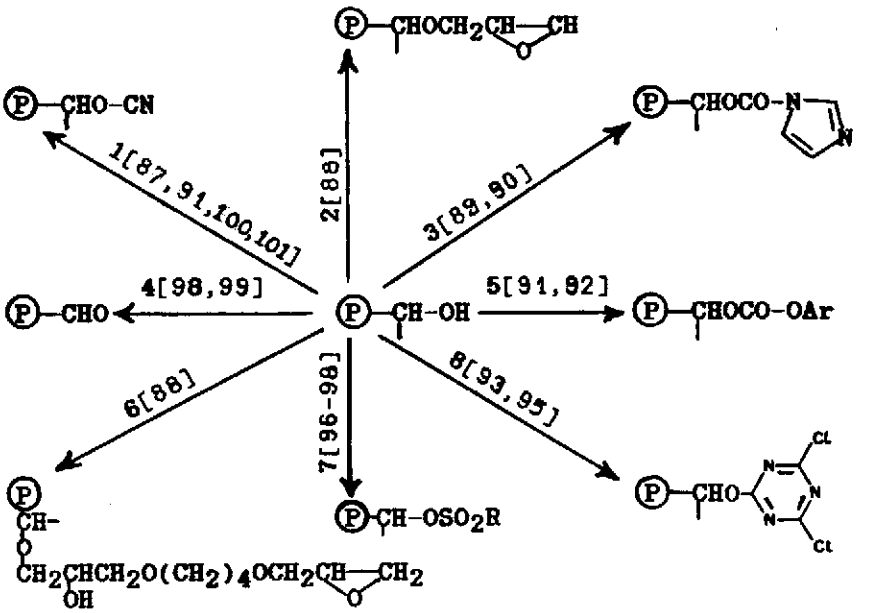


Fig. 12. Preparation of succinyl-activated polysaccharides.



1 = CNBr or O_2N-⟨⟩-OCN; 2 = $ClCH_2CH(O)CH_2$;

3 = Carbonyldiimidazole, 4 = $I\tilde{O}_4$

5 = Cl-CO-OAr (Ar, see Figure 12);

6 = $H_2C(O)CHCH_2O(CH_2)_4OCH_2CH(O)CH_2$;

7 = $ClSO_2R$, R = CH_2CF_3, -⟨⟩-CH_3, N=N-⟨⟩-$N(CH_3)_2$;

8 = Cyanuric chloride (2,4,6-trichloro-1,3,5-triazine)

Fig. 13. Activation of polysaccharide gels.

the use of these reagents is offset by substantially increased reagent cost and additional labour.

Activation of polysaccharide gels by cyanogen bromide and most other reagents indicated in Fig. 13 often involves a variety of side-reactions and complications, some of which are outlined in Fig. 14. For example, O-cyanyl activated gels, in addition to hydrolysis, undergo intra-resin transformation with neighbouring (including spatially nearby) hydroxy groups, leading to the formation of cyclic or inter-chain carbonate and imidocarbonate bridges [90].

Activation by sulphonyl chlorides produces primary and secondary sulphonates, depending on the reaction conditions employed. Only primary sulphonates are sufficiently reactive under the mild conditions desired for ligand/enzyme attachment. Residual sulphonate groups on the polymer increase gel hydrophobicity. It is also possible that secondary

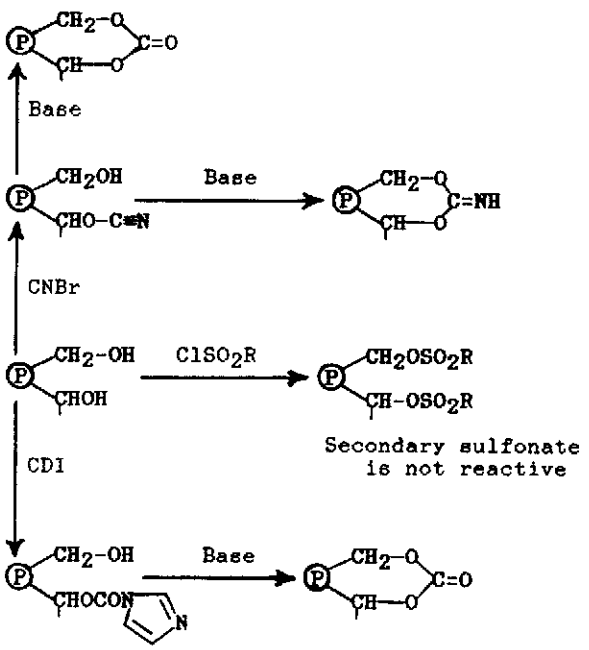


Fig. 14. Possible side-reactions associated with the activation of polysaccharide gels.

sulphonate residues may gradually hydrolyse or react with the soluble or polymer-bound enzyme (or substrate) during the utilization of the gel.

It should also be noted that the reaction of highly functionalized polymer supports with symmetrical difunctional reagents (*e.g.*, diamines or diepoxides) generally leads to extensive intra-resin cross-linking (*cf.* Fig. 9 and ref. 52). The large excess of reagents usually employed for these reactions is not effective because of the intra-resin proximity of the reactive sites. This means that functionalization of polymer supports via symmetrical difunctional reagents may produce gels with lower than expected functionality, higher degrees of cross-linking, increased rigidity and correspondingly altered (reduced) porosity.

5.4. Porous silica and glass beads

Functionalization of siliceous gels is based on the chemistry of surface silanol groups. The silanol function can be derivatized via several reaction pathways [32], including condensation with alcohols and trichloro- and trialkoxysilanes. In particular, reaction with triethoxysilanes [32,102–106] provides a highly versatile route for the production of stable functionalized silica supports in a single step (Fig. 15). Silylating reactions according to Fig. 15 are carried out by refluxing the silica particles in a solution of the reagent in pure toluene [103,104], in toluene contaminated with (traces of) water [104,105] or in aqueous media [106]. In either case, the chemistry of the reaction is complicated because the OH groups may be free or hydrogen bonded, depending on the thermal history of the particles [104,107]. The silylation reaction shown in Fig. 15 is also applicable to metal oxide supports such as titania and zirconia, as reported recently by Truedinger *et al.* [108].

The theoretical silanol content of porous silica varies with porosity and surface area, but is usually about 3–4 mmol (mequiv.) per gram of dry sample. However, most of these OH groups are either buried within the silica grains (isolated small pores), or are otherwise strongly hydrogen bonded and inaccessible. The presence of water in the reaction mixture reduces the level of hydrogen bonding and creates additional silanol sites via hydrolysis of surface Si–O–Si bonds. In this way, the reaction conditions can be empirically adjusted to produce a so-called monolayer functionality of up to about 0.2–0.3 mmol/g. In strictly rigid supports, functionality may also be expressed in units of μmol/m^2.

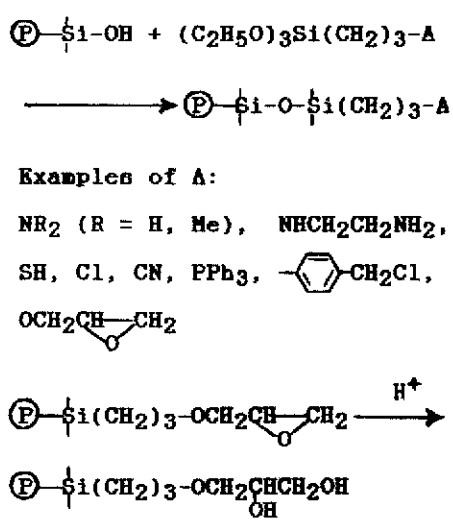


Fig. 15. Derivatization of silica gel via reaction with triethoxysilanes.

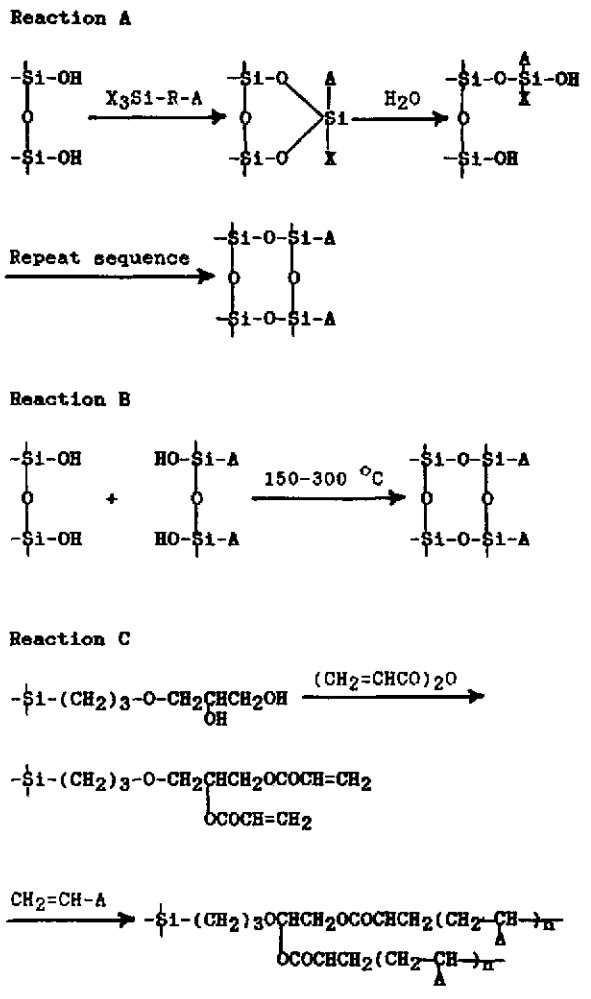


Fig. 16. Preparation of highly functionalized silica gel by surface polycondensation of alkoxy or chlorosilanes (Reaction A), polymeric alkoxysilanes (Reaction B) or surface polymerization of organic monomers (Reaction C).

Once this primary derivatization has been accomplished, the resulting functional groups can be activated or further derivatized as necessary [109,110]. One particularly useful reaction is the acid hydrolysis [106,111] of the oxirane (epoxide) functionality to obtain the corresponding diol derivative. Here again, the accessibility of the initially generated functional groups depends on sample porosity and reaction conditions. Surface modification of colloidal silica particles by organic polymers has been recently reviewed by Ryan [112].

Silica particles with relatively higher degrees of functionality can be produced by forming, or attaching (grafting), polymeric species onto the initially generated functional groups. Three different routes for the formation of polymeric species on porous silica are depicted in Fig. 16.

In the stepwise condensation of chloro- or alkoxysilanes on the silica surface (Fig. 16, Reaction A) [113], the stoichiometry of the reaction is very difficult to control. On the other hand, according to Kirkland and Yates [114], the reaction of silica with preformed polyethoxysilanes (Reaction B) can be easily controlled to obtain a multilayer thickness of about 3–1000 nm. Graft polymerization of vinyl monomers on appropriately functionalized silica surfaces (Reaction C) [115] is essentially similar to the formation of core–shell grafts discussed in Part I. This method usually leads to the formation of cross-linked (entrapped) organic polymer within the pore structure of the silica.

5.5. *Amphiphilic copolymers*

In recent years, a new synthetic approach has been introduced [116–118] whereby both the functionality and the chemical structure of the polymer can be tailored for optimum performance. The method is based on a new class of activated polymer intermediates and the chemistry of active esters (active ester synthesis or leaving group substitution) (Fig. 17) [119–121].

According to the new method, the activating (or leaving) groups on the polymer are displaced by suitably chosen nucleophiles carrying the desired structure (A^1) or functionality (A). As can be seen in Fig. 17, the functional residue (A) is positioned at the end of a spacer arm (B). In addition, the choice of the structural residue (A^1) offers the possibility of "tailoring" the composition of the polymer support for any specific application. In particular, the new method provides a uniquely versatile route for the synthesis of amphiphilic polymer supports carrying the desired functionality (Table 5).

The range of functional groups which can be introduced into the polymer according to Fig. 17 is virtually unlimited. For polymer supports with low degrees of functionality (< 1 mmol/g), the reaction

sequence (1) HA^1, (2) HBA is more convenient, except when HA^1 is a volatile compound (*e.g.* dimethylamine). There is a possibility that a fractionation of the reactive sites generated on the resin initially may be relatively more accessible than those introduced at the end. Accordingly, the reaction sequence (1) HBA, (2) HA^1 should be preferred in principle. However, the practical significance of this differential accessibility would appear to depend on the particular application involved. The possibility of generating reactive sites with relatively low accessibility is of special interest in the design and study of site isolation on the polymer support.

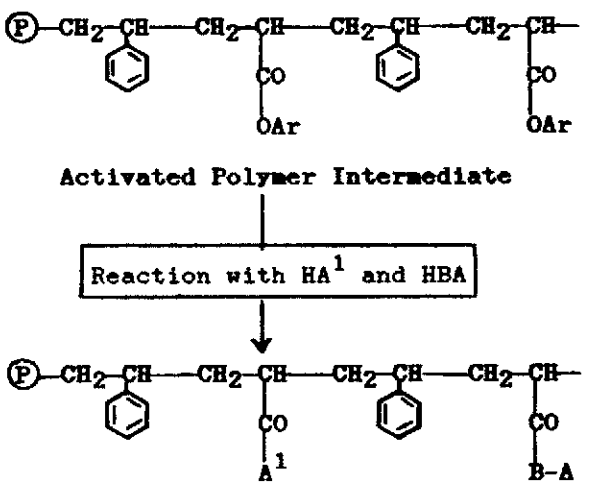
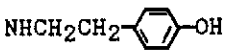
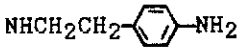
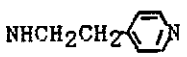
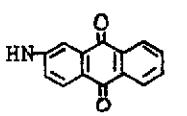


Fig. 17 Synthesis of amphiphilic polymer supports via activated polymer intermediates (active ester synthesis or leaving group substitution) [116–118].

TABLE 5

EXAMPLES OF AMPHIPHILIC POLYMER SUPPORTS AVAILABLE VIA ACTIVE ESTER SYNTHESIS ACCORDING TO FIG. 17

A^1	B–A
$NHCH_2CH_2N(CH_3)_2$	$NHCH_2CH_2N(CH_3)_2$
$NHCH_2CH_2SO_3H$	$NHCH_2CH_2SO_3H$
$NHCH_2CH_2CH_2OH$	$NHCH_2CH_2CH_2OH$
$N(CH_3)_2$	$N(CH_3)_2$
$N(CH_3)_2$	$NHCH_2CH(OH)CH_2OH$
$N(CH_3)_2$	$NH(CH_2)_6OH$
$N(CH_2)_2$	$NH(CH_2)_5COOH$
$N(CH_3)_2$	$NH(CH_2)_6NH_2$
$N(CH_3)_2$	
$N(CH_3)_2$	
$N(CH_3)_2$	$O(CH_2CH_2O)_nOH$
$N(CH_3)_2$	$O(CH_2)_6NHCHO^a$
$N(CH_3)_2$	$O(CH_2)_6PPh_2$
$NHCH_2CH_2CH_2OH$	
$N(CH_3)_2$	

[a] The NHCHO group can be dehydrated to the corresponding isocyano (NC) group, useful for metal complexation or enzyme/ligand attachment by four-component condensation.

6. POLYMER MATRIX AND CHEMICAL STRUCTURE

6.1. Polymer matrix

The term "polymer matrix" is employed here to refer to the macromolecular (or *secondary*) structure of the polymer chains within the individual microbeads. A schematic illustration of this structure for a lightly cross-linked non-porous gel matrix is provided in Fig. 18 [122]. Such a polymer matrix is expected to result from the "random coil" nature of the polymer chains and the conditions under which the microbeads are usually formed.

When a low-cross-linked gel swells in a good solvent (see Tables 3 and 4), the matrix expands and a certain degree of short-range re-organization of the loose and tight chain segments may take place. Long-range conformational changes are, however, prohibited by the cross-link bridges between the

Fig. 18. Schematic presentation of the macromolecular (or matrix) structure of a lightly cross-linked polymer bead.

chains. In addition, extensive non-covalent cross-linking (*e.g.* hydrophobic interaction in polystyrene, or hydrogen bonding in polyacrylamides and polysaccharides) may restrict even the short-range mobility of the chain segments. In other words, polymer supports and gels have a heterogeneous matrix structure which is largely preserved even in the swollen state.

Routine experience indicates that when non-porous gels swell in a "good" solvent to a minimum of about 5 ml/g, all of the polymer-bound reactive sites are usually accessible within a reactivity range of 1–2 orders of magnitude. That is, in fully swollen gels some of the reactive sites on a single polymer microbead may be 10–100 times less reactive than others. In highly cross-linked and porous gels, the degree of site heterogeneity is correspondingly higher. In the extreme case, and where the polymer does not swell, only the surface reactive groups may be accessible. Even some of the surface groups may have reduced accessibility because of strong non-covalent interactions with the neighbouring groups and/or the polymer backbone.

To this end, it should be stressed that the overall efficiency of a given polymer support is not necessarily determined by full site accessibility. In general, chromatographic and catalytic applications of polymer supports and gels require rapid diffusion of the substrate to, and from, the polymer matrix. Here, accessibility of all of the reactive sites is by no means essential. On the other hand, for organic chemical applications, and notably for solid-phase peptide synthesis, it is essential that all of the polymer-bound reactive sites are more or less equally accessible. In this case, high reaction rates are desirable, but not critical. Accordingly, the architecture of the polymer matrix should be designed for either rapid diffusion or maximum site accessibility, depending on the requirement of the intended application.

6.2. Chemical structure

Traditionally, structure–performance relationships in polymer supports and gels are studied on the basis of porosity and surface area (or *tertiary structure*). The significance of the polymer matrix (or *secondary structure*) is also generally recognized, as outlined above. An even deeper level of gel structure, which may critically influence the overall gel performance, is the chemical (or *primary*) structure of the polymer backbone. This level of structure–performance relationship originates from the fact that the "function" of the polymer support, whether in chromatography, catalysis or synthesis, is invariably based on "direct molecular contacts" between the polymer backbone and the soluble substrate.

Experimental evidence on the relationship between the chemical structure of the polymer backbone and overall polymer performance abounds in the literature. However, the significance of these observations is not always appreciated, and hence they may be left "unexplained", or reported as "thermodynamic" or "microenvironment" effects. Notable examples where explanation in terms of chemical structure has been offered include the role of chemical structure in gel permeation chromatography [123], aqueous hydrolysis of species bound to hydrophobic polymer supports [124], polymer-supported hydrogenation catalysts [125,126], and in peptide synthesis [34]. Some of these observations have been briefly reviewed recently [117]. The conclusion is that, in many instances, the overall efficiency of a given gel may depend critically on the chemical compatibility of the polymer backbone with the polymer-bound substrate or the soluble reagent.

6.3. Site accessibility and spacer arm

Functional groups attached to cross-linked polymer matrices usually have reduced accessibility compared with those of analogous low-molecular-weight compounds. This decreased site accessibility is partly due to reduced mobility of the cross-linked chain segments and partly the result of "spatial" hindrance within the cross-linked matrix. The term "spatial" (rather than steric) is employed to emphasize the effect of "through-space" interactions *versus* steric effects of neighbouring residues observed in small molecules.

Both segmental mobility of the polymer chains and spatial hindrance (matrix architecture) are closely related to polymer cross-linking and swellability, but they represent two different aspects of matrix structure. Spatial hindrance cannot be easily measured and quantified, whereas segmental mobility can be quantified by, for example, electron spin resonance [127,128] and NMR spectroscopy [129].

Reduced site accessibility caused by low mobility of the polymer chains can, in principle, be remedied by introducing a spacer arm between the polymer backbone and the functional groups. In practice, the degree by which a given spacer arm may enhance site accessibility depends largely on the size and nature of the soluble reagent. However, a five- or six-bond spacer arm is usually considered useful for most applications of polymer supports, including affinity chromatography, immobilized enzymes and solid-phase synthesis and catalysis.

Typical spacer molecules employed in conjunction with different polymer supports are indicated in Fig. 19. In conventionally produced polymers, the positioning of functional groups at the end of such spacer arms usually involves a multi-step synthesis.

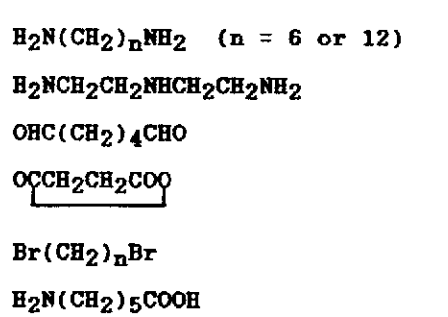


Fig. 19. Structures of typical spacer molecules employed in conjunction with the use of different polymer supports and gels.

For the recently introduced amphiphilic copolymer resins, the desired functionality, already positioned at the end of a four- to nine-bond spacer arm (see Table 5), is introduced into the polymer in a single step.

7. CONCLUSIONS AND GENERAL REMARKS

Beaded polymer supports and gels, including polystyrene, polyacrylamides, polymethacrylates, polysaccharides, porous silica, copoly(styrene acrylamide)s and composites, are produced by various modes of two-phase suspension systems. The main feature of these two-phase systems is the formation of "microdroplets" of the desired monomer or polymer solution, followed by their conversion to the corresponding "microbeads". The conversion of the liquid droplets to solid polymer particles may involve a polymerization or polycondensation process, or it may require solvent extraction or covalent cross-linking of the dissolved polymer.

The size, porosity and surface area of the polymer beads obtained by two-phase suspension processes can be easily controlled by various manufacturing parameters. The relationship between these parameters and bead characteristics is fairly well established for synthetic organic polymers (*e.g.* polystyrene, polymethacrylates and polyacrylamides), but they are less fully documented for polysaccharides and silica gel. An extensive array of chemical reactions and reagents are available for functionalization and activation of various polymer types considered above. However, chemical transformation of polymer supports and gels may often involve undesirable side-reactions, and sufficient care must be exercized to avoid or minimize such complications.

Structure–performance relationships in polymer supports and gels are traditionally studied on the basis of surface area and porosity (*i.e. tertiary structure*). The effect of the polymer matrix (or *secondary structure*) is also being increasingly recognized. However, the "function" of the polymer support, whether in chromatography, catalysis or synthesis, is invariably based on "direct molecular contacts" between the polymer backbone and the soluble substrate. Thus, in addition to surface area, porosity and matrix structure, an appreciation of the chemical (or *primary*) structure of the polymer backbone is suggested to be essential for a better

understanding of the behaviour of polymer supports and gels. By the same token, our knowledge of "chemical structure" and "polymer–solvent–substrate" interactions can be employed to design and tailor polymer supports and gels for optimum performance.

A number of interesting new polymer supports, including inorganic–organic composites, interpenetrating networks, core–shell grafts and amphiphilic copoly(styrene–acrylamide)s with general solvent and substrate compatibility, have been introduced in recent years. Further development of these new materials along the above lines is expected to attract increasing interest in the future.

REFERENCES

1 T. Kremmer and L. Boros, *Gel Chromatography*, Wiley, New York, and Akadémiai Kiadó, Budapest, 1979.
2 R. Epton (Editor), *Chromatography of Synthetic and Biological Macromolecules*, Vols. 1 and 2, Ellis Horwood, Chichester, 1978.
3 J. J. Kirkland (Editor), *Modern Practice of Liquid Chromatography*, Wiley, New York, 1971.
4 F. C. Frank and R. C. Chang, *The Practice of Ion Exchange Chromatography*, Wiley, New York, 1983.
5 C. Calmon, *AIChE Symp. Ser.*, 80 (1984) 84.
6 J. Inczedy, *Analytical Applications of Ion Exchangers*, Pergamon Press, Oxford, 1966.
7 T. C. J. Gribnau, J. Visa and R. J. F. Nivard, *Affinity Chromatography and Related Techniques*, Elsevier, Amsterdam, 1982.
8 P. D. G. Dean, W. S. Johnson and F. A. Middle (Editor), *Affinity Chromatography: a Practical Approach*, IRL Press, Oxford, 1985.
9 L. Jervis, in D. C. Sherrington and P. Hodge (Editors), *Syntheses and Separations Using Functional Polymers*, Wiley, Chichester, 1988, pp. 265–303.
10 S. G. Allenmark, *Chromatographic Enantioseparations*, Ellis Horwood, Chichester, 1988.
11 K. Mosbach (Editor), *Methods in Enzymology*, Vol. 112, Academic Press, New York, 1985.
12 J. Woodward, *Immobilized Cells and Enzymes: a Practical Approach*, IRL Press, Oxford, 1985.
13 R. B. Merrifield, *Makromol. Chem. Makromol. Symp.*, 19 (1988) 31.
14 J. M. Stewart and J. D. Young, *Solid Phase Peptide Synthesis*, Pierce, Rockford, IL, 2nd ed., 1984.
15 W. T. Ford (Editor), *Polymeric Reagents and Catalysts*, American Chemical Society, Washington, DC, 1986.
16 *Pure Appl. Chem.*, 60 (1988) 353.
17 D. C. Sherrington and P. Hodge (Editors), *Syntheses and Separations Using Functional Polymers*, Wiley, Chichester, 1988.
18 A. Warshawsky, in M. Streat and D. Naden (Editors), *Ion Exchange and Sorption Processes in Hydrometallurgy*, Wiley, New York, 1987, pp. 127–225.
19 A. Rembaum and Z. A. Tokes (Editors), *Microspheres: Medical and Biological Applications*, CRC Press, Boca Raton, FL, 1988.
20 K. Nustad, L. Johansen, J. Ugelstadt, T. Elingsen and A. Berge, *Eur. Surg. Res.*, 16, Suppl. 2 (1984) 80.
21 R. Arshady, *J. Chromatogr.*, 586 (1991) 181.
22 R. Arshady and A. Ledwith, *React. Polym.*, 1 (1983) 159.
23 H. Hopff, H. Lussi and E. Hammer, *Makromol. Chem.*, 82 (1965) 175 and 184.
24 F. Wolf and S. Eckert, *Plaste Kautsch.*, 18 (1971) 650 and 890; 19 (1972) 26.
25 T. Allen, *Particle Size Measurement*, Chapman & Hall, London, 2nd ed., 1975.
26 C. D. Scott, *Anal. Biochem.*, 42 (1968) 292.
27 J. V. Dawkins, T. Stone and G. Yeadon, *Polymer*, 18 (1977) 1179.
28 S. M. Ahmed, *Dispersion Sci. Technol.*, 5 (1984) 421.
29 R. Arshady, *Colloid Polym. Sci.*, in preparation.
30 J. W. Hassler, *Purification with Activated Carbon*, Chemical Publishing, New York, 1971.
31 J. S. Mattson and H. B. Mark, *Activated Carbon*, Marcel Dekker, New York, 1971.
32 K. K. Unger, *Porous Silica*, Elsevier, Amsterdam, 1979.
33 N. V. Akshinaskaya, A. V. Kisclev and Y. S. Nikitin, *Russ. J. Phys. Chem.*, 37 (1963) 927 (reported in ref. 32).
34 R. Arshady, *Adv. Mater.*, 3 (1991) 182.
35 H. Galina, N. B. Colaz, P. P. Wiezorek and M. Wojszynska, *Br. Polym. J.*, 17 (1985) 215.
36 W. Heitz, *Adv. Polym. Sci.*, 23 (1977) 1.
37 A. Guyot and M. Bartholin, *Prog. Polym. Sci.*, 8 (1982) 277.
38 J. C. Moore, *J. Polym. Sci., Part A-2*, (1969) 835.
39 D. Horak, Z. Pelzbauer, M. Bleha, M. Ilavsky, F. Svec and J. Kalal, *J. Appl. Polym. Sci.*, 26 (1980) 411.
40 D. Horak, F. Svec, M. Bleha and J. Kalal, *Angew. Makromol. Chem.*, 95 (1981) 109.
41 D. Horak, F. Svec, M. Ilavsky, M. Bleha and J. Kalal, *Angew. Makromol. Chem.*, 95 (1981) 117.
42 J. Coupek, M. Krivakova and S. Pokorny, *J. Polym. Sci., Polym. Symp.*, 42 (1973) 185.
43 R. Ruchel and M. D. Brager, *Anal. Biochem.*, 68 (1975) 415.
44 W. Rolls, F. Svec and J. M. J. Frechet, *Polymer*, 31 (1990) 165.
45 S. Brunauer, P. Emmet and E. Teller, *J. Am. Chem. Soc.*, 60 (1938) 309.
46 E. P. Barret, L. J. Joyner and P. P. Halenda, *J. Am. Chem. Soc.*, 73 (1951) 373.
47 J. Gregg and K. S. W. Sing, *Adsorption, Surface Area and Porosity*, Academic Press, London, 1982.
48 B. H. Davis, *Appl. Catal.*, 10 (1984) 185.
49 M. Brun, J. F. Quinson, R. Blanc, M. Negre, C. Eyrand and M. Bartholin, *Makromol. Chem.*, 182 (1981) 873.
50 R. Arshady, unpublished results; *cf.* ref. 22.
51 R. Arshady, *Makromol. Chem.*, 189 (1988) 1295.
52 R. Arshady, *Colloid Polym. Sci.*, 268 (1990) 948.
53 *Gel Filtration: Theory and Practice*, Pharmacia, Uppsala.
54 J. Peska, J. Stamberg and J. Hradil, *Angew. Makromol. Chem.*, 53 (1976) 73.
55 M. Smrz and J. Viska, in R. Epton (Editor), *Chromatography of Synthetic and Biological Macromolecules*, Vol. 1, Ellis Horwood, Chichester, 1978, pp. 91–108.

56 E. Fenyvesi, B. Szadon, J. Szejtli and F. Tudos, *Ann. Univ. Sci. Budap. Rolando Eotvos Nominatae Sect. Chim.*, 15 (1979) 13.
57 A. V. J. Brough, R. Epton, G. Marr, A. T. Shackley and G. A. Sniezko-Blocki, in R. Epton (Editor), *Chromatography of Synthetic and Biological Macromolecules*, Vol. 1, Ellis Horwood, Chichester, 1978, pp. 70–90.
58 A. V. J. Brough, R. Epton, J. V. McLaren, *Polymer*, 18 (1977) 1208.
59 C. Birr, *Aspects of Merrifield Peptide Synthesis*, Springer, Berlin, 1978.
60 M. Negre, M. Bartholin and A. Guyot, *Angew. Makromol. Chem.*, 106 (1982) 79.
61 D. G. Barer, K. P. Staller and N. A. Peppas, *Ind. Eng. Chem., Prod. Res. Dev.*, 22 (1983) 161.
62 E. A. Grulke, in J. Brandrup and E. H. Immergut (Editors), *Polymer Handbook*, Wiley, New York, 3rd ed., 1989, pp. VII/519–559.
63 J. F. Kennedy and J. M. S. Cabral, *Trans. Met. Chem.*, 11 (1986) 41; 12 (1987) 481.
64 R. W. Siergiej and N. D. Danielson, *Anal. Chem.*, 55 (1983) 17.
65 A. Dias and T. J. McCarty, *Macromolecules*, 18(1985) 1826.
66 R. Millar, D. G. Smith, W. E. Marr and T. R. E. Kressman, *J. Chem. Soc.*, (1963) 218.
67 K. W. Pepper, H. M. Paisely and M. A. Young, *J. Chem. Soc.*, (1953) 4097.
68 M. J. Hudson (Editor), *Recent Developments in Ion Exchange*, Elsevier Applied Science, Barking, 1987.
69 J. A. Malinsky (Editor), *Ion Exchange*, Marcel Dekker, New York, 1969.
70 R. S. Feinberg and R. B. Merrifield, *Tetrahedron*, 30 (1974) 3209.
71 J. T. Sparrow, *Tetrahedron Lett.*, 1975 (1975) 4637.
72 R. E. Barron and J. S. Fritz, *React. Polym.*, 1 (1983) 215.
73 M. J. Farral and J. M. J. Frechet, *J. Org. Chem.*, 41 (1976) 3877.
74 G. A. Crosby, N. M. Weinshenker and H. S. Uh, *J. Am. Chem. Soc.*, 97 (1975) 2232.
75 D. C. Sherrington, in P. Hodge and D. C. Sherrington (Editors), *Polymer-Supported Organic Synthesis*, Wiley, Chichester, 1980, pp. 1–82.
76 J. K. Inman and H. M. Dintzis, *Biochemistry*, 8 (1969) 4047.
77 L. Goldstein, *Methods Enzymol.*, 135 (1987) 90.
78 R. Arshady and I. Ugi, *Polymer*, 31 (1990) 1164.
79 R. Epton, B. L. Hibart and G. Marr, *Polymer*, 16 (1975) 314.
80 R. Arshady, E. Atherton, D. L. J. Clive and R. C. Sheppard, *J. Chem. Soc., Perkin Trans. 1*, (1981) 529.
81 C. K. Narang, K. Brunfeldt and K. E. Norris, *Tetrahedron Lett.*, (1977) 1819.
82 R. Arshady, E. Atherton and R. C. Sheppard, *Tetrahedron Lett.*, 1979 (1979) 1521.
83 R. Arshady, unpublished results.
84 E. A. Peterson and H. A. Sober, *J. Am. Chem. Soc.*, 78 (1956) 751.
85 E. A. Peterson and H. A. Sober, *Biochem. Prep.*, 8 (61) 39 and 43.
86 H. Determan and T. Wieland, *Makromol. Chem.*, 114 (1968) 263.
87 R. Axen, J. Porath and S. Earnback, *Nature (London)*, 214 (1967) 1302.
88 L. Sundberg and J. Porath, *J. Chromatogr.*, 171 (1974) 87.
89 G. S. Bethell, J. S. Ayers, M. T. W. Hearn and W. S. Hancock, *J. Chromatogr.*, 219 (1981) 353 and 361.
90 M. T. W. Hearn, L. E. Harris, G. S. Bethell, W. S. Hancock and J. S. Ayers, *J. Chromatogr.*, 219 (1981) 509.
91 M. Wilchek, *Angew. Makromol. Chem.*, 123/124 (1984) 175.
92 L. Drobnik, J. Labsky, H. Kudlvasrova, U. Sandec and F. Svec, *Biotechnol. Bioeng.*, 24 (1982) 487.
93 G. Kay and E. M. Crook, *Nature (London)*, 216 (1967) 514.
94 C. Longstoff, in P. D. G. Dean, W. S. Johnson and F. A. Middle (Editors), *Affinity Chromatography: a Practical Approach*, IRL Press, Oxford, 1985, pp. 37–38.
95 J. S. Ayers, M. J. Peterson, B. E. Sheerin and G. S. Bethel, *J. Chromatogr.*, 294 (1984) 195.
96 K. Nilsson and K. Mosbach, *Methods Enzymol.*, 104 (1984) 56.
97 W. H. Scouten and W. van der Tweel, in I. M. Chaiken, M. Wilchek and J. Parikh (Editors), *Affinity Chromatography and Biological Recognition*, Academic Press, New York, 1983, pp. 229–302.
98 N. Weliky and H. H. Weetall, *Immunochemistry*, 2 (1965) 293.
99 T. Miron and M. Wilchek, *J. Chromatogr.*, 215 (1981) 55.
100 J. Kohn and M. Wilchek, *Chem. Anal. (Warsaw)*, 66 (1983) 599.
101 J. Kohn and M. Wilchek, *Biochem. Biophys. Res. Commun.*, 107 (1982) 878.
102 E. Grushka (Editor), *Bonded Stationary Phases in Chromatography*, Ann Arbor Sci. Publ., Ann Arbor, MI, 1974.
103 H. Engelhardt and D. Mathes, *J. Chromatogr.*, 142 (1977) 311.
104 M. Lynn and A. M. Filbert, in E. Grushka (Editor), *Bonded Stationary Phases in Chromatography*, Ann Arbor Sci. Publ., Ann Arbor, MI, 1974, pp. 1–11.
105 K. G. Allum, R. D. Hancock, I. V. Howell, S. McKenzie, R. C. Pikethly and P. J. Robinson, *J. Organomet. Chem.*, 87 (1975) 203.
106 R. R. Walters in P. D. G. Dean, W. S. Johnson and F. A. Middle (Editors), *Affinity Chromatography: a Practical Approach*, IRL Press, Oxford, 1985, pp. 35–39.
107 U. Kittelmann and K. Unger, *Prog. Colloid Polym. Sci.*, 67 (1980) 19.
108 U. Truedinger, G. Mueller and K. K. Unger, *J. Chromatogr.*, 535 (1990) 111.
109 P.-O. Larsson, M. Glad, L. Hansson, S. Ohlson and K. Mosbach, *Adv. Chromatogr.*, 21 (1983) 41.
110 W. Parr and M. Novotny, in E. Grushka (Editor), *Bonded Stationary Phases in Chromatography*, Ann Arbor Sci. Publ., Ann Arbor, MI, 1974, p. 173.
111 F. E. Regnier and R. Noel, *J. Chromatogr. Sci.*, 14 (1976) 316.
112 K. Ryan, *Chem. Ind. (London)*, (1988) 359.
113 R. E. Majors and H. J. Hopper, *J. Chromatogr. Sci.*, 12 (1974) 767.
114 J. J. Kirkland and P. C. Yates, *US Pat.*, 3 722 181 (1973) and 3 795 313 (1974).
115 J. P. J. Verlaan, J. P. C. Bootsma and G. Challa, *J. Mol. Catal.*, 14 (1982) 1211.

116 R. Arshady, B. S. R. Reddy and M. H. George, *Polymer*, 27 (1986) 69.
117 R. Arshady, in preparation.
118 R. Arshady, *Makromol. Chem.*, 185 (1984) 2387.
119 J. Pless and R. A. Biossonas, *Helv. Chim. Acta*, 46 (1963) 1609.
120 M. Bodanszky, *Principles of Peptide Synthesis*, Springer, Heidelberg, 1984.
121 M. Bodanszky, *The Practice of Peptide Synthesis*, Springer, Heidelberg, 1984.
122 R. Arshady, *Chim. Ind. (Milan)*, 70, No. 9 (1988) 70.
123 D. J. Harmon, in R. Epton (Editor), *Chromatography of Synthetic and Biological Macromolecules*, Vol. 1, Ellis Horwood, Chichester, 1978, pp. 122–145.
124 R. Arshady, *Angew. Makromol. Chem.*, 106 (1982) 191.
125 J. K. Stille, *J. Makromol. Sci. Chem., Part A2*, 13–14 (1984) 1689.
126 N. Takashi, H. Imai, C. A. Bertelo and J. K. Stille, *J. Am. Chem. Soc.*, 100 (1978) 264.
127 S. L. Regan, *Macromolecules*, 8 (1975) 689.
128 S. L. Regan, *J. Am. Chem. Soc.*, 96 (1974) 5275.
129 W. T. Ford, M. Peryasamy and H. O. Spivey, *Macromolecules*, 17 (1984) 2881.