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PACKINGS AND STATIONARY PHASES IN PREPARATIVE COLUMN LIQUID CHROMATOGRAPHY*

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1 INTRODUCTION

The rapid development of biotechnology and the requirements placed on the purity of biopharmaceutical products have drastically increased the importance of preparative and scaled-up column liquid chromatography in purification and isolation processes. The substances concerned include complex organic molecules, *e.g.*, antibiotics, and proteins of a molecular mass above 10 kdaltons, *e.g.*, enzymes and monoclonal antibodies of concentrations between 0.1 g dm^{-3} and 1 μg dm^{-3} in the starting mixture. Particular emphasis has been placed on the isolation of expensive materials with selling prices exceeding US $1000 kg^{-1}.

In order to purify the product as cost-effectively as possible, methods are sought that provide a high specificity and a high recovery. One of the key aspects currently influencing the effectiveness of column liquid chromatographic techniques relates to the evaluation of improved and tailored support materials, capable of highly selective separations and flexible with respect to process optimization. Although the theoretical concepts of preparative chromatography provide clear directives regarding the support properties, practical implications, *e g.*, fouling, regeneration, stability and purity, are equally important.

This paper attempts to analyse the decisive features of supports that control the target quantities of the purification processes. It provides an up-to-date survey of the media applied in the various modes of column liquid chromatography and discusses general aspects concerning the choice of appropriate phase systems.

2 ROLE OF PACKINGS AND STATIONARY PHASES IN THE OPTIMIZATION OF LOADABILITY AND THROUGHPUT

The established relationships between loadability, throughput, maximum product of resolution and sample input, and the chromatographic process parameters provide a rational basis for discussing the effects of the support and stationary phase properties. The performance (with respect to the specified purity and yield of a desired product) of an isolation process is determined by the loadability and the throughput[1,2]. Loadability, defined as the maximum sample input, $Q_i(\max)$, corresponding to certain requirements with respect to resolution and peak shape, is given by

$$Q_i(\max) = 2\pi^{\frac{1}{2}} A \, \varepsilon_m \, L \, (C_{i,m})^{\max}(1+k'_i)N_i^{-\frac{1}{2}} \tag{1}$$

where A is the column cross-section, ε_m the porosity of the chromatographic bed, L the column length, $(C_{i,m})^{\max}$ the maximum $C_{i,m}$ value of the elution profile at the column outlet, k' the capacity factor of the solute i and N_i the plate number of the column

Eqn. 1 implies that the maximum sample mass to be injected depends on the maximum solute concentration in the mobile phase at the column outlet. $(C_{i,m})^{\max}$ was shown to be a primary function of the shape of the solute isotherm, and hence controlled by the type of phase system employed[1,2]. It is also evident from eqn. 1 that $Q_i(\max)$ increases with increasing solute capacity factor and with decreasing plate number of the column. Studies by De Jong and co-workers[1] have demonstrated that the specific loadability of a given column, expressed as sample mass injected per

gram of packing, increases with decreasing plate number; a so-called loadability borderline was established where the column dispersion played a negligible role and the elution profile was dictated by the isotherm non-linearity broadening[1].

The throughput, T_i, defined as the mass of product isolated per unit time at a desired purity, is given by

$$T_i = Q_i(\max)/t_{Ri} \quad (2)$$

where t_{Ri} is the retention time of solute i

When the mass of product isolated equals 95% of Q_i(max), assuming a solute sampling interval of $4\sigma_{ti}$ seconds, eqn. 2 becomes

$$T_i = f_v \cdot 2\pi^{\frac{1}{2}} \cdot 0.95\, (C_{i,m})^{\max} \cdot \frac{1 + k'_i}{1 + k'_l} \cdot N_i^{-\frac{1}{2}} \quad (3)$$

where f_v is the volume flow-rate, k'_i and k'_l are the capacity factors of solute i and the last eluting compound l and N_i is the required plate number.

On optimizing the throughput $[(C_{i,m})^{\max}]$, the solute capacity factor and the plate number of the column play decisive roles in addition to the eluent flow-rate. Columns packed with large particles and operated at high flow-rates appear to favour a high throughput.

Whereas in analytical separations the aim is the optimum resolution of an extremely small feed volume, the main concern in preparative isolation is to obtain a maximum of the product of the chromatographic resolution, R_{ji}, and the sample input, Q_i[3].

The sample input, Q_i, is equal to

$$Q_i = C_i^0\, v_0 \quad (4)$$

where C_i^0 is the initial concentration of solute i and v_0 the feed volume. Assuming

$$v_0^2 \gg (1+k'_i)^2/(k'_i\, N_i) \quad (5)$$

the product $R_{ji}\, Q_i$ approaches the limiting value

$$\lim R_{ji}\, Q_i = (k'_j - k'_i)\, v_m \cdot \frac{C_i^0}{\psi} \quad (6)$$

where k'_i and k'_j are the capacity factors of the solutes i and j (k'_j being larger than k'_i), v_m is the volume of mobile phase in the column and ψ is a factor characterizing the shape of the input peak.

Eqn. 6 implies that where there is a large difference between the peak maxima of solutes i and j, the concentration of the sample can be increased at a large feed volume to maximize $R_{ji}\, Q_i$. The peak positions of solutes i and j with column overloading are again controlled by the shape of the isotherm.

In conclusion, highly selective phase systems are essential in preparative work in order to achieve a high loadability and throughput.

3. CRITERIA FOR THE EFFICIENT OPERATION OF PACKINGS AND PHASE SYSTEMS IN PREPARATIVE CHROMATOGRAPHY

The equations presented in the preceding section provide fundamental guidelines for exploiting simple preparative separation schemes. In practice, sample mixtures containing solutes at various concentration ratios have been applied in binary and ternary solvent systems in both isocratic and gradient elution modes. Thus, a reliable estimate of loadability should be based on data taken from isotherms of multicomponent solutions rather than from isotherms of single solutes[4]. When the solutes to be purified differ in their polarity, the peak profiles at high loading can be disturbed by displacement phenomena; these might cause a loss or improvement in resolution at increased loadability[5]. Peak profiles have also been known to be affected by the mode of injection, *i.e.*, by the design of the injection device[1,2,6,7]. The successful application of packings in practice depends on a series of criteria that are not included in the basic equations and hence will be discussed below.

3.1. Bed stability and flow resistance

Bed stability is a necessary precondition for high-resolution separations. In preparative chromatography, the columns are longer and wider than in routine analytical work, packed with larger particles and operated at higher flow-rates. The relationship between the linear eluent velocity, u, and the column pressure drop, Δp, is given by

$$u = \frac{\Delta p K_0}{\eta L} = \frac{\Delta p d_p^2}{\Phi L \Phi} \tag{7}$$

where K_0 is the chromatographic permeability, η the eluent viscosity, L the column length, d_p the particle diameter of the packing and Φ the flow resistance factor[8]. As a result, a linear relationship holds between the eluent flow-rate and the column pressure drop. Semi-rigid and soft gels, however, deviate from the expected linearity owing to their compressibility.

One way to overcome possible bed compression of semi-rigid gels is to use stacked columns[9]. Devices for adjusting the bed height of preparative columns have also been constructed. Shrinkage and/or swelling of the column bed may also occur with soft and semi-rigid organic gels, depending on the degree of cross-linking and on the eluent composition. With small-bore columns the bed is supported by the column walls, whereas with large-bore columns this stabilizing influence is negligible. Thus, distinctly different flow pressure characteristics are obtained for columns varying in diameter, under otherwise constant conditions. By increasing the column length the weight of the packing is also increased, which concurrently raises the hydrostatic pressure. Therefore, when employing large bed heights the particles should possess sufficiently high mechanical stability. Another phenomenon that must be taken into account is the abrasive action of the flow on the particles; this generates fines which

then block the column outlet. In order to avoid all of these problems, spherical particles are to be preferred to irregularly shaped particles, as has been the practice in the operation of fixed-bed catalytic reactors and large-scale adsorption columns Spherical particles with adequate particle uniformity and acceptable size distribution provide the most dense and stable column bed.

3.2. Chemical resistance and purity

None of the packings in column liquid chromatography behaves in a totally inert manner towards the eluent and the solutes. Rather, they exhibit a limited lifetime, associated with degradation by dissolution, chemical surface reactions, ageing and other processes. On using bonded silicas, traces of organosilicon compounds might be released from the packing when the bonding reaction has not been carried out properly, or conditions might be chosen that favour a cleavage of bonded groups. Monomers might dissolve from polymer gels. All these phenomena will impair the purity of the products to be separated, presenting a serious problem in the isolation of biopharmaceutical products. The elimination of traces of impurities by subsequent purification procedures is extremely time consuming and costly. For this reason, the utmost care is advised when choosing the packing material.

3.3. Solute accessibility

This criterion is of lesser relevance in the separation of low-molecular-weight compounds; however, it is highly important in the isolation of large molecules, particularly proteins and enzymes. A series of studies (for comparison see ref. 10) demonstrated that a major part of the internal surface of a packing is not fully utilized for the separation of large molecules by interaction chromatography; this was attributed to limited surface access. In other words, the main concern is to achieve a surface that is fully accessible to a solute of defined size. This finding led to the development and propagation of so-called wide-pore materials for the separation of biopolymers by column liquid chromatography. It was also shown that even a sufficiently large external surface of small non-porous particles is adequate to achieve an excellent resolution of biopolymers, *e.g.*, by reversed-phase gradient HPLC[10]. These concepts have found widespread application in the continuing design of appropriate macroporous packings.

3.4. Mass recovery and bioactivity

Because of the irreversible adsorption of solutes in chromatographic operation, there is bound to be a certain loss of mass. Hence, a complete mass recovery of the solute in the preparative phase system in operation is required for the complete isolation of the product. There are various methods, and the application of a radiolabelled solute is the most reliable for an assessment of mass recovery. In the isolation of biologically active solutes, the aim is to collect the product without any loss in its bioactivity, *e.g.*, enzymatic activity. Partial unfolding or denaturation of a biopolymer in chromatographic processes is known to be attributable to eluent-mediated and/or surface-induced dynamic effects[11]. The extent of conformational changes of

a given solute, *e.g* , a protein or enzyme, is also observed to be a function of the residence time in a given column. Therefore, maintenance of the biological activity of a solute is specifically dependent on the phase system applied and on the operating conditions.

3.5. Fouling

Fouling is a well known phenomenon in the operation of heterogeneous industrial catalysts It is understood to involve deposition of certain by-products or dust, impairing the catalytic activity and/or selectivity. Coke formation on catalysts in cracking reactions of hydrocarbons is one example. Fouling is also caused by microparticulate matter (fines introduced by the feed). In chromatographic terms, fouling of the surface might take place through preferential adsorption or reaction of active components of the sample mixture. Colloidal fines or particulates from hydrolysates might be deposited in the pores or between the interstices of the packing particles, mainly at the column top, and cause significant changes in column permeability. In the separation of biologically active substances, another type of fouling is microbial contamination, which can be prevented by mobile phase additives and other precautionary measures[12]

3.6. Regeneration[12]

After a period of time, when the resolution or other parameters are observed to decline noticeably, the column should be regenerated to restore its quasi-original state. Regeneration is achieved by washing the column with a solution or solvent of high solvent strength relative to the packing. Often a series of solvents are applied, depending on the compatibility with the eluent. In this way, soluble deposits and impurities adsorbed by the packing are displaced, and the column is returned to a quasi-virgin state. For silicas and their bonded phases, polar or non-polar solvents are applied. Polymer gels are preferably washed with acidic or alkaline solutions. The time required to regenerate a column is dependent on the particle size and the pore size. Packings consisting of large particles have longer diffusion path lengths than microparticles, at constant pore size, eluent and given solute. At constant particle size, diffusion is also a function of the pore size. Packings with small pores require a more extensive period of regeneration than large pore packings.

3.7. Cost

Packings for use in large-scale chromatography should be available at an economical price. This price is determined by the particle shape, the particle size and size distribution, the degree of purity, the extent of surface modification, etc. Spherical particles are about five times more expensive than angular particles; this is due to the specific technology applied for beading. With decreasing average particle size, size classification processes are increasingly reflected by the cost. This is illustrated by the price difference (by a factor of 10) between 10- and 100-μm particles. Narrow cuts are more difficult to produce than those with a broad particle size distribution. Specially purified chemicals must be employed for the manufacture of highly pure

packings, differing from the usual process with technical reagents. A high purity is seldom achieved by extraction of technical-grade packings.

Chemical bonding of adsorbents and supports raises the price by a factor of two for common silanization or other reactions. Specific bonding reagents, such as for the synthesis of chiral and affinity packings, further increase the price.

4 RELEVANCE OF PHYSICAL AND CHEMICAL STRUCTURE PARAMETERS OF PACKINGS IN PREPARATIVE CHROMATOGRAPHY

As chromatographers take no part in the manufacture of packings, they ought to be able to judge the suitability of a packing for a given separation on the basis of the specifications given by the manufacturer. We shall first discuss the physical structure parameters as they are broadly applicable independent of the mode of LC, whereas chemical structure parameters are specific to the type of phase system applied and the solute to be purified.

4.1. Physical structure parameters

Table 1 lists the physical structure parameters of packings, together with their definitions[13,14].

4.1.1 Particle shape

Beaded packings of uniform shape are preferred to angular packings in order to achieve a highly dense and stable column bed, thereby avoiding the abrasion that causes fines, and helping to maintain a high mechanical stability in large columns. These merits must, however, be paid for by the higher price of the spherical packing.

4.1.2. Particle size and size distribution

The average particle diameter of packings in preparative and process chromatography ranges from 20 to 200 μm. Packings with $d_p < 20$ μm are essentially applicable in analytical HPLC and are employed when high plate numbers are required Packings with d_p between 40 and 60 μm provide a good compromise between column pressure drop, Δp, plate number, N, and analysis time, t_c. For optimization of resolution and efficiency in HPLC, see refs. 15 and 5. A narrow particle size distribution with a $d_{p_{90}}/d_{p_{10}}$ ratio of 1.5–2.0 of the cumulative distribution is adequate for the generation of a stable and efficient column.

4.1.3. Specific pore volume, v_p

Except for the size exclusion mode, where v_p determines the phase ratio V_s/V_m of the column, and hence the resolution, the specific pore volume of a packing is of minor importance. For silicas it ranges from 0.5 to 1.5 ml g^{-1}.

4.1.4. Specific surface area, a_s

The specific surface area of a packing controls the capacity and retention of solutes in all interaction modes in column liquid chromatography. This holds for both parent and bonded packings. As packings differ in their packing density, ρ_P, from 0.2 to 0.8 g ml^{-1}, the specific surface area in m^2 g^{-1} must be multiplied by the

TABLE 1

PHYSICAL STRUCTURE PARAMETERS OF SUPPORTS AND PACKINGS[14]

Term	*Symbol*	*Definition*
Particle shape		Angular, spherical
Particle size	d_p	Particle diameter defined according to the method of determination (see Table 4)
Average particle size	d_{p50} d_{pm}	Average particle diameter at 50% of the cumulative distribution (median), d_{p50} or the most frequent average particle diameter of the relative distribution (mode), d_{pm}
Particle size distribution	d_{psd}	Number, volume, weight or surface area distribution
Specific pore volume	v_p	Uptake in ml of liquid per unit mass or unit volume of packing to fill the internal pores
Micropore volume	v_p(micro)	v_p of pores of p_d < 2 nm
Mesopore volume	v_p(meso)	v_p of pores of 2 < p_d < 50 nm
Macropore volume	v_p(macro)	v_p of pores of p_d > 50 nm
Specific surface area	a_s	Internal and external surface area per unit mass or volume of packing
Pore shape		Assumed to be cylindrical in most instances
Pore diameter	p_d	Width of the pore of a given shape
Micropores		p_d < 2 nm
Mesopores		2 < p_d < 50 nm
Macropores		p_d > 50 nm
Hydraulic pore diameter	p_{dn}	Ratio of 4 times the specific pore volume divided by the specific surface area
Kelvin pore diameter	p_{dK}	Pore diameter according to the Kelvin equation
Washburn pore diameter	p_{dW}	Pore diameter according to the Washburn equation
Interstitial column porosity	ε_0	Ratio of intraparticle column volume to total geometric column volume
Internal column porosity	ε_p	Ratio of interparticle column volume to total geometric column volume
Total column porosity	ε(total)	$\varepsilon(\text{total}) = \varepsilon_0 + \varepsilon_p$
Average pore diameter	p_{d50} p_{dm}	Average pore diameter at 50% of the cumulative distribution (median), p_{d50} or the most frequent average pore diameter of the relative distribution (mode), p_{dm}
Pore diameter distribution	p_{dd}	Distribution of v_p or a_s as a function of the average pore diameter

packing density in order to obtain the effective surface area per millilitre of column volume[16]. a_s values of 10–200 m^2 ml^{-1} are sufficient for improving retention. Larger surface areas, generated by pores < 4 nm in diameter, have a number of disadvantages such as slow kinetics, slow regeneration and easy fouling.

4.1.5. Pore diameter, p_d

A pore diameter of about 10 nm accounts for the majority of separations of solutes of M < 2 kdaltons. For larger molecules, pore diameters of *ca.* 30 nm and more are preferable in order to overcome the low diffusivities. Another advantage of large-pore packings is attributed to the improved surface homogeneity compared with small-pore packings. They also permit more rapid and complete regeneration. The pore size distribution usually spans one decade of pore diameter. As above, small pores of p_d < 4 nm should be absent.

Adjustment of the desired physical structure parameters of a packing through the manufacturing process creates fewer problems than the appropriate design of the surface chemistry in terms of retention, selectivity, stability, etc.

4.2. Chemical structure parameters[14]

Grouped according to their bulk composition, packings include inorganic materials and a variety of organic gels (see Table 2). In the last 5 years there have been successful attempts to manufacture packings with improved properties and higher batch-to-batch reproducibility. In general, the following criteria relate to retention and selectivity. type(s) of surface functional groups and their respective interactions

TABLE 2

SURVEY OF TYPES OF PACKINGS IN PREPARATIVE AND PROCESS CHROMATOGRAPHY ACCORDING TO THEIR BULK COMPOSITION

1 Inorganic materials	
Oxides	(silica, alumina, magnesia)
Carbonates	(magnesium carbonate)
Silicates	(magnesium silicate, alumosilicates)
Carbon	
Phosphates	(zirconium phosphate, calcium phosphate)
2 Organic gels	
Cellulose	
Agarose	
Dextran	
Polyacrylamide	
Polyamide	
Poly(hydroxyethyl methacrylate)	
Styrene–divinylbenzene copolymers	
Poly(ethylene glycol dimethacrylate)	
Poly(vinyl alcohol)	
Poly(vinyl acetate)	
3 Chemically bonded silicas	
Reversed-phase silicas	
≡SiR	R = methyl
	ethyl
	n-butyl
	n-hexyl
	n-octyl
	n-octadecyl
	phenyl
Polar bonded silicas	
≡Si(R)R′	R′ = diol
	amino
	nitro
	cyano
	biospecific ligands (low to high molecular weight)
4 Composites	
Silica/polymer gels	

with solutes; density of functional groups at the surface; distribution of functional groups across the particle; accessibility of surface functional groups to solutes; and type of matrix.

An inspection of the surface functionality of packings indicates a broad variety of groups from polar to non-polar, acidic and basic with graduated pK values, and with various polar functionality. A common feature is that packings often bear more than a single type of surface functional group, as is the case for chemically bonded phases. Depending on the type of parent matrix, either hydrophobic or hydrophilic interactions are observed to contribute to solute retention. The ligand density of bonded groups ranges from maximum, which is rarely achieved, to low densities. Functional groups bonded as a monolayer exhibit distinct changes in their structure, depending on solvation, with respect to chain length. As an alternative to the monolayer type, bonding is performed in such a way that a multilayer is formed, with internal cross-linking. Specific functional groups are bonded at the outer layer in a diluted state, or spaced by extended hydrophilic or hydrophobic groups, in order to control solute accessibility. All the above cases reflect the current design of packings for bonded phase adsorption, ion-exchange and affinity chromatography.

5 SURVEY OF COMMERCIAL PACKINGS IN PREPARATIVE AND PROCESS-SCALE CHROMATOGRAPHY

This survey deals exclusively with packings with an average particle diameter $d_p > 20$ μm, as otherwise all analytical packings would also have to be listed. For information on analytical packings, see refs. 17 and 18.

5.1 Adsorbents (see Table 3)

Among inorganic adsorbents, silica-based materials, available in graduated particle sizes and size distributions, are the most widely used[19,20]. Most of the products are of technical grades and were not specifically designed for high-quality preparative separations. The specific surface areas range from 300 to 500 m^2 g^{-1}. At packing densities of about 0 5 g ml^{-1}, a_s values of 150–250 m^2 per ml column volume are achieved. Some commercial products are of the macroporous type, with surface areas lower than 50 m^2 g^{-1}. In order to improve the limited stability at high pH, attempts were made to dope silica with zirconia and alumina. However, all these manipulations remain fairly inefficient as long as the bulk structure is amorphous. In this context, it is worth mentioning that purely crystalline silica, *e.g.*, silicalite I, or a crystalline silica-rich pentasil type of zeolite with an SiO_2/Al_2O_3 ratio above 100, are insoluble in strongly alkaline media[21]. Unfortunately they contain such small micropores that they cannot be applied in liquid chromatography.

Bonded phases include *n*-octyl- and *n*-octadecyl-modified silicas for reversed-phase chromatography and a small number of the polar bonded silica types.

There has been little progress in terms of purity, defined phase composition and pore size in the manufacture of aluminas and other materials for chromatography. The traditional aluminas, adjusted to a certain acidity and basicity, are still on the market[20].

The same situation is met with organic-based adsorbents. The classical ones,

TABLE 3

SURVEY OF COMMERCIAL ADSORBENTS IN COLUMN LIQUID CHROMATOGRAPHY (d_p > 20 μm)

Abbreviations used: * = angular, o = spherical, b = bulk material, c = column.

Type	*Name*	*Supplier*	*Form*	d_p *(μm)*	a_s *(m^2g^{-1})*	p_d *(nm)*	*Comments*
Silica	Matrex Silica Media	Amicon, Grace	*,b	20, 30, 50, 105	540 320 320	6 10 25	pH of a 5% (w/w) suspension 6.0–6.5
Silica	XWP 500 Å	Amicon, Grace	*,b	30–100	60	50	
	XWP 1000 Å	Amicon, Grace	*,b	30–100	40	100	
	XWP 1500 Å	Amicon, Grace	*,b	30–100	28	150	
Silica	Bakerbond normal-phase silica gel	Baker	*,b	32–62, 50–200	500	6	
Silica	Bio-Sil HA	Bio-Rad	*,b	< 100	–	–	
	Bio-Sil A	Bio-Rad	*,b	20–44, 80–150	–	–	
Silica	ICN silica	ICN	*,b	0–63, 32–100, 63–100, 63–200, 100–200, 200–500	550	6	
	ICN silica N	ICN	*,b	18–32, 32–63	550	6	
Silica	KC-Mikroperl M	Kali Chemie	o,b	30–60	210 130 76 65 55 47 38	10 18 27 34 43 54 68	
	KC-Mikroperl L	Kali Chemie	o,b	100–200			
Silica	Nucleosil 100–30	Macherey–Nagel	o,b	30 ± 10	350	10	
	Polygosil 60	Macherey–Nagel	*,b	15–25, 25–40, 40–63, 63–100	500	6	
	Polygosil 100	Macherey–Nagel	*,b	25–40, 40–63	300	10	

(Continued on p. 238)

TABLE 3 *(continued)*

Type	*Name*	*Supplier*	*Form*	d_p *(μm)*	a_s *(m^2g^{-1})*	p_d *(nm)*	*Comments*
Silica	Kieselgel 40	Merck	*,b	63–200 200–500	> 500	4 4	pH of a 10% (w/w) suspension ≈7, content of Fe 0.02%, Cl 0.03%
	Kieselgel 60	Merck	*,b	< 63 63–200 200–500	500 500 500	6 6 6	
	Kieselgel 100	Merck	*,b	63–200 200–500	250 250	10 10	
	Kieselgel 60	Merck	*,b	15–40 40–63 63–100	500	6	Narrow size classification
	Kieselgel 60 reinst	Merck	*,b	63–200	500	6	Highly pure grade, Fe 0.002%, Cl 0 008%
Silica	LiChroprep Si 40	Merck	*,b	15–25 40–63	–	–	
	LiChroprep Si 60	Merck	*,b,c	15–25 40–63	–	–	
	LiChroprep Si 100	Merck	*,b	15–25 40–63	–	–	
Silica	Kieselguhr	Merck	*,b	–	–	–	
Silica	Porasil A	Millipore, Waters	o,b	37–75 75–125	300–500		
	Porasil B	Millipore, Waters	o,b	37–75 75–125	140–230		
Silica	Prep-PAK 500 silica	Millipore, Waters		55–105	–	–	
Silica	Vydac TP silica Vydac HS silica	Separations Group	*,b,c o,b,c	20–30 20–30	80 500	30 8	
Silica	LPS-1 LPS-2	Whatman Whatman	*,b *,b	13–24 37–53	250 450		

Reversed-phase silica	Matrex silica Media C_8 C_{18}	Amicon, Grace	*,b	20, 30 50, 105	–	6 10 25	*n*-Octyl and *n*-octadecyl groups bonded
Reversed-phase silica	Bakerbond Methyl Ethyl Butyl, Hexyl Octyl, Octadecyl Phenethyl Diphenyl	Baker	*,b	40	~500	~6	
Reversed-phase silica	ICN silica RP-8 ICN silica RP-18	ICN ICN	*,b *,b	18–32, 32–63 18–32, 32–63	– –	– –	*n*-Octyl groups bonded *n*-Octadecyl groups bonded
Reversed-phase silica	Nucleosil 30 C_{18} Polygosil 60 C_8 Polygosil 60 C_{18}	Macherey–Nagel Macherey–Nagel Macherey–Nagel	o,b *,b *,b	30 ± 10 25–40 40–63 25–40, 40–63	– – –	– – –	*n*-Octadecyl groups bonded *n*-Octyl groups bonded *n*-Octadecyl groups bonded
Reversed-phase silica	Prep-PAK Vydac C4	Millipore, Waters	c	30	–		*n*-Butyl groups bonded
	Prep-PAK Vydac C_{18}	Millipore, Waters	c	30	–	–	*n*-Octadecyl groups bonded
Reversed-phase silica	Prep-PAK 500 C_{18}	Millipore, Waters	c	55–105	–	–	*n*-Octadecyl groups bonded
Reversed-phase silica	Bondapak C_{18}/ Porasil B	Millipore, Waters	o,b	37–75	–	–	*n*-Octadecyl groups bonded
Silica, silanized	Kieselgel 60 silanized	Merck	*,b	63–200	-	6	Dimethylsilyl functional groups
Reversed-phase silica	Kieselgel 60 silanized	Merck	*,b	63–200	500	6	Dimethylsilyl groups bonded
	LiChroprep RP-2 LiChroprep RP-8	Merck Merck	*,b *,b,c	25–40 5–20, 15–25, 25–40, 40–63	– –	– –	Dimethylsilyl groups bonded *n*-Octyl groups bonded

(Continued on p. 240)

TABLE 3 *(continued)*

Type	*Name*	*Supplier*	*Form*	d_p *(µm)*	a_s *(m^2g^{-1})*	p_d *(nm)*	*Comments*
	LiChroprep RP-18	Merck	*,b,c	5–20, 15–25, 25–40, 40–63	–	–	*n*-Octadecyl groups bonded
Reversed-phase silica	Vydac 201 HS		o,b,c	20–30	–	–	*n*-Octadecyl groups bonded
	Vydac 201 TP		*,b,c	20–30	–	–	*n*-Octadecyl groups bonded
	Vydac 214 TP		*,b,c	20–30	–	–	*n*-Butyl groups bonded
	Vydac 218 TP		*,b,c	20–30	–	–	*n*-Octyl groups bonded
	Vydac 219 TP		*,b,c	20–30	–	–	Diphenyl groups bonded
Reversed-phase silica	Synchroprep RP-P C_{18}	Synchrom	b,c	30	–	–	*n*-Octadecyl groups bonded
	Synchroprep RP-P C_8	Synchrom	b,c	30	–	–	*n*-Octyl groups bonded
	Synchroprep RP-P C_4	Synchrom	b,c	30	–	–	*n*-Butyl groups bonded
	Synchroprep RP-P C_1	Synchrom			–	–	Methyl groups bonded
Reversed-phase silica	LRP-1	Whatman	*,b	13–24	–	–	*n*-Octadecyl groups bonded
	LRP-2	Whatman	*,b	37–53	–	–	*n*-Octadecyl groups bonded
Polar bonded silicas	Bakerbond normal-phase	Baker	*,b	40	500	6	
	Diol						Diol groups bonded
	Cyanopropyl						Cyano groups bonded
Chiral phases	DNBPG-ionic	Baker	*,b	40			(*R*)-N-3,5-Dinitrobenzoylphenylglycine
	DNB Leu-kovalent	Baker	*,b	40	–	–	(*S*)-N-3,5-Dinitrobenzoylleucine
Polar bonded silicas	LiChroprep Diol	Merck	*,b,c	25–40, 40–63	–	–	Diol groups bonded
	LiChroprep NH_2	Merck	*,b	25–40, 40–63	–	–	Amino groups bonded
	LiChroprep CN	Merck	*,b,c	40–63	–	–	Cyano groups bonded
Alumina	Neutral alumina AG 7	Bio-Rad	*,b	–	–	–	pH 6.9–7.1
	Basic alumina AG 10	Bio-Rad	*,b	–	–	–	pH 10.0–10.5
	Acid alumina AG 4	Bio-Rad	*,b	–	–	–	pH 3.5–4.5

Alumina	ICN alumina N	ICN	*,b	18–32, 32–63	200	6	pH 7 5
	ICN alumina A	ICN	*,b	18–32	200	6	pH 4 5
	ICN alumina B	ICN	*,b	18–32	200	6	pH 10
Alumina	Aluminiumoxid 60, aktiv basisch	Merck	*,b	63–200	–	6	pH 9.0 ± 0.5
	Aluminiumoxid, 90, aktiv basisch	Merck	*,b	63–200	–	9	pH 9.0 ± 0 5
	Aluminiumoxid 90, aktiv neutral	Merck	*,b	63–200	–	9	pH 7.3 ± 0.5
	Aluminiumoxid 90, aktiv sauer	Merck	*,b	63–200	–	9	pH 4 0 ± 0.5
	Aluminiumoxid 150, basisch	Merck	*,b	63–200	–	15	pH 9 0 ± 0.5
Alumina	LiChroprep Alox T	Merck	*,b	25–40	–	–	Basic alumina
Magnesium silicate	Florisil	Merck	*,b	75–150, 150–200	–	–	Bulk composition MgO SiO_2 = 15 · 85
Cellulose	Matrex Cellufine Cellulose	Amicon, Grace	*,b	–		–	
Cellulose	Matrex Cellufine AM	Amicon, Grace	o,b		–		
Dextran	Matrex Cellufine Dextran	Amicon, Grace	b	–		–	
Agarose	Matrex Cellufine Agarose	Amicon, Grace	b	–		–	
Macroporous styrene–divinyl-benzene copolymers	Bio-Beads SM-2	Bio-Rad	o,b		300	9	
	Bio-Beads SM-4	Bio-Rad	o,b	–	725	4	
Acrylic ester	Bio-Beads SM-7	Bio-Rad	o,b	–	450	9	
Cellulose, acetylated	Cellulose CEL AC-30X	Macherey–Nagel	*,b	15–85			
	Cellulose CEL AC-40X	Macherey–Nagel	*,b	15–35			

(Continued on p. 242)

TABLE 3 *(continued)*

Type	*Name*	*Supplier*	*Form*	d_p *(μm)*	a_s *(m^2g^{-1})*	p_d *(nm)*	*Comments*
Polyamide	PA-6/2032	Macherey–Nagel	*,b	20–32	–	–	
Cellulose	Cellulose Makrokristallin Avicel	Merck	*,b	20–100	–	–	
Styrene–divinyl-benzene copolymers	Amberlite XAD-2	Merck	o,b	20–50			
	Amberlite XAD-4	Merck	o,b	20–50			
acrylic ester	Amberlite XAD-7	Merck	o,b	20–50			
	Amberlite XAD-8	Merck	o,b	20–50			
Crosslinked agarose	Phenyl-Sepharose	Pharmacia	o,b				
	Octyl-Sepharose	Pharmacia	o,b				

such as cellulose, dextran, agarose and styrene–divinylbenzene copolymers, still dominate, while the pressure-stable, highly cross-linked polymers with large pore sizes have not yet been introduced as preparative packings.

5.2. Size exclusion packings (see Table 4)

A wide variety of organic-based gels of graduated exclusion limits are on the market, based on classical styrene–divinylbenzene copolymers, polyacrylamide and methacrylate copolymers with different rigidities. Porous silicas and porous glasses are available as inorganic SEC packings. They carry appropriate functional groups, depending on the application, for the SEC of synthetic materials or biopolymers.

5.3. Ion-exchange packings (see Table 5)

Basic organic and inorganic packings serve as starting materials for ion exchangers, available as strong to weak cation and anion exchangers. The materials differ in their effective ion-exchange capacities and, relatedly, in the degree of cross-linking and pore size.

Major emphasis is placed on ion exchangers for use in protein separations, *i.e.*, mostly anion exchangers. For this purpose, calcium phosphate or hydroxyapatite are also employed.

5.4. Affinity packings (see Table 6)

The largest number of packings, with an enormous diversity with regard to biospecific affinity, is found in affinity chromatography. Packings are categorized into activated packings and those ready-for-use, carrying group-specific ligands and more biospecific ligands. Activated affinity packings offer the choice of preparing the desired material for a given separation problem through a coupling reaction. Typical activator groups are N-hydroxysuccinimide ester, carboxyl and carboxymethyl groups, amino and oxirane groups, imidocarbonate and 2-pyridyldisulphide groups.

6 KEY ASPECTS IN CHOOSING A PHASE SYSTEM FOR PREPARATIVE AND PROCESS CHROMATOGRAPHY

Chromatographic separation techniques are commonly applied as a last step in purification processes. As is apparent from the preceding discussion, a high chromatographic resolution is necessary in order to achieve the desired purity and yield at an economically acceptable cost, and hence this feature governs the choice of a suitable LC mode. The type(s) of phase system to be selected is primarily determined by the chemical structure properties of the desired product relative to those of the contaminants and by-products. Obviously, the LC modes differ in their selectivity, such as size and shape (size exclusion chromatography), charge (ion-exchange chromatography), polarity and type of functional groups (adsorption chromatography), isoelectric point (chromatofocusing), hydrophobic character (reversed-phase and hydrophobic interaction chromatography), biological affinity (affinity chromatography)[22]. It is often the case that a single-step procedure based on one LC mode

TABLE 4

SURVEY OF COMMERCIAL SIZE EXCLUSION PACKINGS ($d_p > 20$ μm)

For abbreviations, see Table 3

Type	*Name*	*Supplier*	*Form*	d_p (μm)	p_d (nm)	a_s ($m^2 g^{-1}$)	*Molecular weight fractionation range (daltons)*
Silica	Fractosil 200	Merck	*,b	40–63/63–125	18	150	< 50000 (polystyrenes)
	Fractosil 500	Merck	*,b	40–63/63–125	50	50	<400000 (polystyrenes)
	Fractosil 1000	Merck	*,b	40–63/63–125	100	20	<1000000 (polystyrenes)
	Fractosil 2500	Merck	*,b	63–125	250	8	
	Fractosil 5000	Merck	*,b	63–125	500	3	
	Fractosil 10000	Merck	*,b	63–125	1000	1 5	
	Fractosil 25 000	Merck	*,b	63–125	2500	0.6	
Silica with a carbohydrate layer	SynChroprep GPC 25 000	SynChrom	*,b	60–120			
	SynChroprep GPC 10000	SynChrom	*,b	60–120			
	SynChroprep GPC 5000	SynChrom	*,b	60–120			
	SynChroprep GPC 2500	SynChrom	*,b	60–120			
Silica with polymerized polyamine coating	SynChroprep CATSEC 25000	SynChrom	*,b	60–120			
	SynChroprep CATSEC 10000	SynChrom	*,b	60–120			
	SynChroprep CATSEC 5000	SynChrom	*,b	60–120			
	SynChroprep CATSEC 2500	SynChrom	*,b	60–120			
Porous glass	CPG-100	Pierce	*,b	37–74	10	170	1000–30000 (dextrans)
	CPG-240	Pierce	*,b	37–74	24	130	2500–125000 (dextrans)
	CPG-500	Pierce	*,b	37–74/125–177	50	70	11000–350000 (dextrans)
Porous glass with bonded glycerol phase	CPG-40 Glykophase	Pierce	b	37–74	4		1000–8000 (dextrans)
	CPG-100 Glykophase	Pierce	b	37–74	10		1000–30000 (dextrans)

	CPG-200 Glykophase	Pierce	b	37–74	20	2500–125 000 (dextrans)
	CPG-460 Glykophase	Pierce	b	37–74	46	11 000–350 000 (dextrans)
Cellulose	Matrex Cellufine GC 100	Amicon, Grace	o,b	< 10		10 000–60 000 (globular proteins)
	Matrex Cellufine GC 200	Amicon, Grace	o,b	< 20		10 000–120 000 (globular proteins)
	Matrex Cellufine GC 700	Amicon, Grace	o,b	< 70		10 000–400 000 (globular proteins)
	Matrex Cellufine GCL 2000	Amicon, Grace	o,b	< 300		10 000–2 000 000 (globular proteins)
Agarose	Bio-Gel A-0 5 m	Bio-Rad	o,b	40–80/80–150 150–300		10 000–500 000 (globular biomolecules)
	Bio-Gel A-1 5m	Bio-Rad	o,b	40–80/80–150 150–300		10 000–1 500 000 (globular biomolecules)
	Bio-Gel A-5 m	Bio-Rad	o,b	40–80/80–150 150–300		10 000–5 000 000 (globular biomolecules)
	Bio-Gel A-15 m	Bio-Rad	o,b	40–80/80–150 150–300		40 000–15 000 000 (globular biomolecules)
	Bio-Gel A-50 m	Bio-Rad	o,b	80–150/150–300		100 000–50 000 000 (globular biomolecules)
	Bio-Gel A-150 m	Bio-Rad	o,b	80–150/150–300		1 000 000–150 000 000 (globular biomolecules)
Agarose	Sepharose 2B	Pharmacia	o,b	60–200		7 10^4–4 10^7 (proteins)/10^5–2×10^7 (polysaccharides)
	Sepharose 4B	Pharmacia	o,b	60–140		6 10^4–2 10^7 (proteins)/3 10^4–5 10^6 (polysaccharides)
	Sepharose 6B	Pharmacia	o,b	45–165		10^4–4 10^6 (proteins)/10^4–10^6 (polysaccharides)
Cross-linked agarose	Sepharose CL-2B	Pharmacia	o,b	60–200		See Sepharose
	Sepharose CL-4B	Pharmacia	o,b	60–140		See Sepharose
	Sepharose CL-6B	Pharmacia	o,b	45–165		See Sepharose
Cross-linked dextran	Sephadex G-10	Pharmacia	o,b	40–120		< 700 (globular proteins)/< 700 (dextrans)
	Sephadex G-15	Pharmacia	o,b	40–120		< 1500 (globular proteins)/< 1500 (dextrans)
	Sephadex G-25	Pharmacia	o,b	10–40/20–80 50–150/100–300		1000–5000 (globular proteins)/100–5000 (dextrans)
	Sephadex G-50	Pharmacia	o,b	10–40/20–80 50–150/100–300		1500–30 000 (globular proteins)/500–10 000 (dextrans)
	Sephadex G-75	Pharmacia	o,b	10–40/40–120		3000–80 000 (globular proteins)/1000–50 000 (dextrans)
	Sephadex G-100	Pharmacia	o,b	10–40/40–120		4000–150 000 (globular proteins)/1000–50 000 (dextrans)
	Sephadex G-150	Pharmacia	o,b	10–40/40–120		5000–300 000 (globular proteins)/1000–150 000 (dextrans)
	Sephadex G-200	Pharmacia	o,b	10–40/40–120		5000–600 000 (globular proteins)/1000–200 000 (dextrans)

(Continued on p. 246)

Type	*Name*	*Supplier*	*Form*	d_p *(µm)*	p_d *(nm)*	a_s *(m^2g^{-1})*	*Molecular weight fractionation range (daltons)*
Cross-linked dextran	Sephacryl S-200 superfine	Pharmacia	o,b	40–105			$5\cdot10^3$–$2.5\cdot10^5$ (proteins)/$1\cdot10^3$–$8\cdot10^4$ (polysaccharides)
	Sephacryl S-300 superfine	Pharmacia	o,b	40–105			$1\cdot10^4$–$1.5\cdot10^6$ (proteins)/$2\cdot10^3$–$4\cdot10^5$ (polysaccharides)
	Sephacryl S-400 superfine	Pharmacia	o,b	40–105			$2\cdot10^4$–$8\cdot10^6$ (proteins)/$1\cdot10^4$–$2\cdot10^6$ (polysaccharides)
	Sephacryl S-500 superfine	Pharmacia	o,b	40–105			$4\cdot10^4$–$2\cdot10^7$ (polysaccharides)
	Sephacryl S-1000 superfine	Pharmacia	o,b	40–105			$5\cdot10^5$–10^8 (polysaccharides)
Polyethylene glycol dimethyl acrylate	Fractogel PGM 2000	Merck	*,b	32–63/63–100 100–250			<2000 (polyethylene glycol)
Polyacrylamide	Bio-Gel P-2	Bio-Rad	o,b	<40, 40–80			100–1800 (globular biomolecules)
	Bio-Gel P-4	Bio-Rad	o,b	<40. 40–80, 80–150			800–4000 (globular biomolecules)
	Bio-Gel P-6	Bio-Rad	o,b	<40, 40–80, 80–150, 150–300			1000–6000 (globular biomolecules)
	Bio-Gel P-10	Bio-Rad	o,b	<40 40–80, 80–150, 150–300			1500–20 000 (globular biomolecules)
	Bio-Gel P-30	Bio-Rad	o,b	<80, 80–150, 150–300			2500–40 000 (globular biomolecules)
	Bio-Gel P-60	Bio-Rad	o,b	<80, 80–150, 150–300			3000–60 000 (globular biomolecules)
	Bio-Gel P-100	Bio-Rad	o,b	<80, 80–150, 150–300			5000–100 000 (globular biomolecules)
	Bio-Gel P-150	Bio-Rad	o,b	<80, 80–150, 150–300			15 000–150 000 (globular biomolecules)
	Bio-Gel P-200	Bio-Rad	o,b	<80, 80–150, 150–300			30 000–200 000 (globular biomolecules)
	Bio-Gel P-300	Bio-Rad	o,b	<80, 80–150, 150–300			60 000–400 000 (globular biomolecules)
	Bio-Gel P-6 DG (desalting gel)	Bio-Rad	o,b	90–180			1000–6000 (globular biomolecules)
Hydrophilic polymer	Toyopearl G 6000 PW	Toyo Soda	o,c	25 ± 5			40 000–8 000 000 (polyethylene glycol) 100 000–20 000 000 (dextran)
Hydrophilic cross-linked vinyl polymer	Fractogel TSK HW-40	Merck	*,b	25–40, 32–63, 50–100			100–10 000 (globular proteins)
	Fractogel HW-50	Merck	*,b	25–40, 32–63			500–200 000 (globular proteins)
	Fractogel HW-55	Merck	*,b	25–40, 32–63			1000–1 000 000 (globular proteins)
	Fractogel HW-65	Merck	*,b	25–40, 32–63			50 000–5 000 000 (globular proteins)
	Fractogel HW-75	Merck	*,b	25–40, 32–63			500 000–50 000 000 (globular proteins)

Hydrophilic cross-linked vinyl polymer	Toyo Pearl HW-40	Toyo Soda		Product identical with Fractogel TSK HW-40	
	Toyo Pearl HW-50	Toyo Soda		Product identical with Fractogel TSK HW-50	
	Toyo Pearl HW-55	Toyo Soda		Product identical with Fractogel TSK HW-55	
	Toyo Pearl HW-65	Toyo Soda		Product identical with Fractogel TSK HW-65	
	Toyo Pearl HW-75	Toyo Soda		Product identical with Fractogel TSK HW-75	
Styrene–divinyl-benzene copolymer	Styragel 60 Å	Millipore, Waters	*,b,c	<37, 37–75	10–500 (polystyrenes)
	Styragel 100 Å	Millipore, Waters	*,b,c	37–75	50–1500 (polystyrenes)
	Styragel 200 Å	Millipore, Waters	*,c	37–75	50–4000 (polystyrenes)
	Styragel 500 Å	Millipore, Waters	*,b,c	37–75	100–10 000 (polystyrenes)
	Styragel 10^3 Å	Millipore, Waters	*,c	37–75	200–30 000 (polystyrenes)
	Styragel 10^4 Å	Millipore, Waters	*,c	37 75	5000–6 10^5 (polystyrenes)
	Styragel 10^5 Å	Millipore, Waters	*,c	37–75	50 000–4 10^6 (polystyrenes)
	Styragel 10^6 Å	Millipore, Waters	*,c	37–75	2 10^5–1 10^7 (polystyrenes)
	Styragel 10^7 Å	Millipore, Waters	*,c	37–75	5 . 10^5–2 . 10^7 (polystyrenes)
Styrene–divinyl-benzene copolymer	HN-X 2.00 4 00/6 00/6 25/ 6 50/6 75/7.00/ 7 25/7 50/7 75/ 8 00/8 25/8 50/ 8 75/9 00/9 50/ 10 00/11 00/12 00/ 15 00/20 00/25 00/ 30 00/35 00 (% cross-linkage)	Hamilton	o,b	<30, 50	
Styrene–divinyl-benzene copolymer	Bio-Beads S-X1	Bio-Rad	b	40–80	600–14 000 (polystyrenes)
	Bio-Beads S-X2	Bio-Rad	b	40–80	100–2 700 (polystyrenes)
	Bio-Beads S-X3	Bio-Rad	b	40–80	<2000 (polystyrenes)
	Bio-Beads S-X4	Bio-Rad	b	40–80	<1400 (polystyrenes)
	Bio-Beads S-X8	Bio-Rad	b	40–80	<1000 (polystyrenes)
	Bio-Beads S-X12	Bio-Rad	b	40–80	< 400 (polystyrenes)

(Continued on p. 248)

TABLE 5

SURVEY OF COMMERCIAL ION EXCHANGERS ($d_p > 20$ μm)

Type	*Name*	*Supplier*	*Form*	d_p *(μm)*	*Ion-exchange capacity (mequiv ml^{-1})*	*Comments*
Cellulose-based ion exchangers	Matrex Cellufine AM	Amicon, Grace	o,b	–	0 18	DEAE anion exchanger, low to medium molecular weight solutes
	Matrex Cellufine AH	Amicon, Grace	o,b	–	0 14	DEAE anion exchanger medium to high molecular weight solutes
	Matrex Cellufine CM	Amicon, Grace	o,b	–	0 21	Carboxymethyl cation ion exchanger
Cross-linked agarose-based ion exchanger	DEAE-Biogel A	Bio-Rad	o,b		0 02	
	CM-Biogel A	Bio-Rad	o,b		0 02	
Acrylic polymer-based cation exchanger	Bio-Rex 70	Bio-Rad	o,b		1 5	
Styrene–divinylbenzene copolymer-based ion exchanger	Aminex A-4	Bio-Rad	o,b	20 ± 4	1 7	Strong cation exchanger
	Aminex Q-150 S	Bio-Rad	o,b	28 ± 7	1 7	Strong cation exchanger
	Aminex Q-15 S	Bio-Rad	o,b	22 ± 3	1 7	Strong cation exchanger
	Aminex 50 W-X4	Bio-Rad	o,b	25 ± 5		
				32 5 ± 1 5	1 7	Strong cation exchanger
	Aminex A-14	Bio-Rad	o,b	20 ± 3	1,2	Strong cation exchanger
Sulphonated styrene–divinylbenzene copolymer for carbohydrate analysis	HPX-42 A	Bio-Rad	o,c	25		Ag form
	HPX-42 C	Bio-Rad	o,c	25		Ca form
Styrene–divinylbenzene-based ion exchangers						
Strong cation exchanger (SO_3H)	HC-X, 200 up to HC-X 35.00	Hamilton	o,b	20 25		
Strong anion exchanger [$N(CH_3)_3Cl$]	HA-X 4 up to HA-X 10	Hamilton	o,b	20–25	–	
Cellulose-based weak anion exchanger (DEAE)	DE 52	Pierce	b		1 0	
Cellulose-based weak cation exchanger (carboxymethyl)	CM 52	Pierce	b		1 0	
Styrene–divinylbenzene copolymer-based cation exchangers	Amberlyst	Merck	o,b	20–50		

	Lewatit SP 1000	Merck	o,b	60–150		
	Amberlite OG-501	Merck	o,b	100–200		
	Amberlite IRC 50	Merck	o,b	20–50		
	Amberlite IR-120	Merck	o,b	20–50		
	Lewatit S 1080	Merck	o,b	60–150		
Styrene–divinylbenzene copolymer-based anion exchangers	Amberlite IRA-400	Merck	o,b	20–50		
	Lewatit M 5080	Merck	o,b	20–50		
	Amberlyst A-26	Merck	o,b	20–50		
	Lewatit MP 5080	Merck	o,b	60–150		
	Amberlite IR-45	Merck	o,b	20–50		
	Amberlyst A-21	Merck	o,b	20–50		
	Lewatit MP 7080	Merck	o,b	60–150		
Polymer-based weak anion exchanger	Fractogel TSK DEAE 650	Merck	*,b	25–50 49–90	0.10±0 02	Based on TSK HW-65
Polymer-based weak ion exchanger	Fractogel TSK CM 650	Merck	*,b	25–50 45–90	0 10±0 02	Based on TSK HW-65
	Accell QAE	Millipore, Waters	b b	37–55		
	Accell CM	Millipore, Waters		37–55		
Cross-linked dextran	DEAE-Sephadex	Pharmacia	o,b	40–125 (dry)	3.0–4 0	
	QAE-Sephadex	Pharmacia	o,b	40–125 (dry)	2 6–3 4	
	CM-Sephadex	Pharmacia	o,b	40–125 (dry)	4.0–5 0	
	SP-Sephadex	Pharmacia	o,b	40–125 (dry)	2 0–2 6	
Cross-linked agarose	DEAE-Sepharose	Pharmacia	o,b	45–165 (wet)	0.13–0 17	
	CM-Sepharose	Pharmacia	o,b	45–165 (wet)	0 10–0 14	
Cellulose	DEAE-Sephacel	Pharmacia	o,b	40–160	0.17	
Silica-based ion exchanger	Bakerbond ion exchanger WAX	Baker	*,b	40	1 1	
	Aminopropyl WAX	Baker	*,b	40	1 4	
	Diamino SAX	Baker	*,b	40	0 6	
	Quaternary Amine WCX	Baker	*,b	40	0 9	
	Carboxylated					

(Continued on p. 250)

TABLE 5 (*continued*)

Type	*Name*	*Supplier*	*Form*	d_p (μm)	*Ion-exchange capacity (mequiv ml⁻¹)*	*Comments*
	SCX aliphatic sulphonic acid	Baker	*,b	40	0.8	
	SCX aromatic sulphonic acid	Baker	*,b	40	0.9	
Silica-based ion exchanger	SynChroprep AX 300	SynChrom	b,c	30	–	
	SynChroprep Q 300	SynChrom	b,c	30	–	
	SynChroprep CM 300	SynChrom	b,c	30		
	SynChroprep S 300	SynChrom	b,c	30		
Porous glass based ion exchanger	CPG/CM Glycophase	Pierce	*,b	74–125		
	CPG/DEAE Glycophase	Pierce	*,b	74–125		
	CPG/QAE Glycophase	Pierce	*,b	74–125		
	CPG/SP Glycophase	Pierce	*,b	74–125		
Calcium phosphate (hydroxy-apatite)	Bio-Gel HT	Bio-Rad	b	–	10 mg g^{-1} BSA	
	Bio-Gel HTP	Bio-Rad	b	–	10 mg g^{-1} BSA	
Calcium phosphate	Hydroxylapatit sphärisch	Merck	o,b	75–180		Bulk composition 38.3% Ca, 19.7% P

TABLE 6

SURVEY OF COMMERCIAL AFFINITY PACKINGS (d_p > 20 μm)

Type	*Name*	*Supplier*	*Form*	*dp/μm*	*Activator group or ligand*	*Binding capacity*	*Ligand specifity*
Activated affinity suports							
Cross-linked agarose	Affi-Gel 10	Bio-Rad	o,b		N-Hydroxysuccinimide ester on 10-atom, neutral, hydrophilic spacer	15 μmole ml^{-1}	Ligands with primary amino groups
	Affi-Gel 15	Bio-Rad	o,b		N-Hydroxysuccinimide ester on 15-atom, alkaline spacer	15 μmole ml^{-1}	Ligands with primary amino groups
	CM Bio-Gel A	Bio-Rad	o,b		Carboxymethyl group (carbodiimide method)	20 μmole ml^{-1}	Ligands with terminal amino groups
	Affi-Gel 202	Bio-Rad	o,b		Carboxyl group on 10-atom, hydrophilic spacer (carbodiimide method)	20 μmole ml^{-1}	Ligands with primary amino groups
	Affi-Gel 102	Bio-Rad	o,b		Amino group on 6-atom, hydrophilic spacer (carbodiimide method)	15 μmole ml^{-1}	Ligands with carboxyl groups
Poly-acryl-amide	Aminoethyl Bio-Gel P-2	Bio-Rad	o,b	40–80	aminoethyl group (carbodiimide method)	1 mequiv g^{-1} (dry)	Ligands with carboxyl groups
	Aminoethyl Bio-Gel P-150	Bio-Rad	o,b	80–150	aminoethyl group (carbodiimide method)	1 mequiv. g^{-1} (dry)	Ligands with carboxyl groups
Hydro-philic vinyl polymer	Fractogel TSK AF-Epoxy 650	Merck	*,b	32–63	O–CH$_2$–CH–CH$_2$ (epoxide, –CH–CH$_2$ bridged by O)		
	Fractogel TSK AF-Amino 650	Merck	*,b		-O–CH$_2$–CHOH–CH$_2$NH$_2$		
	Fractogel TSK AF-CDI 650	Merck	*,b	32–63	O–CO–N (imidazole ring)		
	Fractogel TSK AF-NCS 650	Merck	*,b	32–63	–NCS		
Cellulose	Aminododecyl-cellulose	Merck	*,b		Amino group on C-12 spacer (carbodiimide method)	0.1 mequiv ml^{-1}	–COOH
	Aminododecyl-cellulose succinyliert	Merck	*,b		Succinimide on C-12 spacer	0 1 mequiv. ml^{-1}	–NH_2

(Continued on p 252)

TABLE 6 *(continued)*

Type	*Name*	*Supplier*	*Form*	*dp/μm*	*Activator group or ligand*	*Binding capacity*	*Ligand specifity*
	Carboxymethyl-cellulosehydrazid	Merck	*,b		Carboxymethyl hydrazide	0 1 mequiv ml $^{-1}$	$-NH_2$
Acrylic polymer	Eupergit C	Rohm Pharma	o,b	30, 150, 250	Oxirane group		
Agarose	CNBr-activated Sepharose 4B	Pharmacia	o,b	60–140	Imidocarbonate groups		$-NH_2$
	AH-Sepharose 4B	Pharmacia	o,b	60–140	Amino group on C-6 spacer	6–10 μmole ml^{-1}	$-COOH$
	CH-Sepharose 4B	Pharmacia	o,b	60–140	Carboxyl group on C-5 spacer (carbodiimide required)	10–14 μmole ml^{-1}	$-NH_2$
	Activated CH-Sepharose 4B	Pharmacia	o,b	60–140	N-Hydroxysuccinimide ester on C-5 spacer	5–7 μmole ml^{-1}	$-NH_2$
	Epoxy-activated Sepharose 6B	Pharmacia	o,b	45–165	Oxirane group on hydrophilic spacer	15–20 μmole ml^{-1}	$-NH_2$, $-OH$, $-SH$
	Activated thiol-Sepharose 4B	Pharmacia	o,b	60–140	2-Pyridyldisulphide on glutathione spacer	1 μmole ml^{-1}	Surface SH groups (also for covalent chromatography)
	Thiopropyl-Sepharose 6B	Pharmacia	o,b	45–165	2-Pyridyldisulphide on hydroxypropyl spacer	20 μmole ml^{-1}	Surface SH groups, heavy metals, various double bonds, alkyl and aryl halides (also for covalent chromatography)
Cross-linked agarose	Reacti-Gel (6X)	Pierce	*,b	45–165 hydrated	Imidazolylcarbamate	> 50 μmole ml^{-1}	$-NH_2$
Cross-linked dextran	Reacti-Gel (25DF)	Pierce	*,b	20–80 dry	Imidazolylcarbamate	> 100 μmole ml^{-1}	$-NH_2$
Polystyrene	Hydrazide beads	Pierce	o,b nonporous	0 25 in	Hydrazide (glutaraldehyde method)	3 μmole per bead	$-NH_2$
	Alkylamine beads	Pierce	o,b nonporous	0.25 in	Hexylamine (succinic anhydride method)	3 μmole per bead	$-NH_2$

	Sanger reagent beads	Pierce	o,b nonporous	0.25 in.	1-Fluoro-2,4-dinitrobenzene on hexylamine spacer	3 μmole per bead	$-NH_2$
Acrylic polymer	Reacti-Gel (GF-2000)	Pierce	b		Imidazolylcarbamate	> 50 μmole ml^{-1}	$-NH_2$
Hydrophilic vinyl polymer	Reacti-Gel (HW-65 F)	Pierce	*,b		Imidazolylcarbamate	> 50 μmole ml^{-1}	$-NH_2$
Porous glass with bonded glycerol phase	CPG/CDI-activated Glycophase	Pierce	*,b	74–125	Imidazolylcarbamate	> 50 μmole ml^{-1}	$-NH_2$
	CPG/aminoacryl	Pierce	*,b	125–177	Aromatic amino group		–COOH
	CPG/aminopropyl	Pierce	*,b	125–177	Aminopropyl		–COOH
	CPG/carboxyl	Pierce	*,b	125–177	Carboxyl group on 10 Å alkyl spacer		$-NH_2$
	CPG/long chain alkylamine	Pierce	*,b	125–177	Amino groups on long alkyl spacer		–COOH
	CPG/NHS glycol phase	Pierce	*,b	125–177	N-Hydroxysuccinimide		$-NH_2$
	CPG/stable diazonium salt	Pierce	*,b	125–177	Diazonium borofluoride on aromatic amine spacer		Phenols, aromatic amines
	CPG/thiol	Pierce	*,b	125–177	SH on 10 Å alkyl spacer		–SH, –Hg
Polyacrylamide–agarose gel	Act-Ultrogel AcA 22	IBF/LKB	*,b	60–140	Glutaraldehyde (Michael addition to the trimer)		$-NH_2$
Polyacrylamide–agarose gel with 7% Fe_3O_4	Act-Magnogel AcA 44	IBF/LKB	*,b	60–140	Glutaraldehyde (Michael addition to the trimer)		$-NH_2$
Polyacrylamide–agarose gel with 7% Fe_3O_4	AC-Ultrogel AcA 34	IBF/LKB	*,b	60–140	Carboxyl group on C-5 spacer		$-NH_2$
	HMD-Magnogel AcA 44	IBF/LKB	*,b	60–140	Amino group on C-6 spacer		–COOH

(Continued on p. 254)

TABLE 6 *(continued)*

Type	*Name*	*Supplier*	*Form*	*dp/μm*	*Activator group or ligand*	*Binding capacity*	*Ligand specifity*
Affinity media with bonded ligands							
Silica	Bakerbond MAb	Baker	*,b,c	40			Mouse monoclonal IgG
hydrophilic polymer	Fractogel TSK AF-blue	Merck	*,b	32–63	Triazine dyes (coupled to activated Fractogel TSK HW-65)	11.6 mg ml^{-1} for BSA	Proteins
	Fractogel TSK AF-red	Merck	*,b	32–63	Triazine dyes (coupled to activated Fractogel TSK HW-65)	11.6 mg ml^{-1} for BSA	Proteins
	Fractogel TSK AF-green	Merck	*,b	32–63	Triazine dyes (coupled to activated Fractogel TSK HW-65)	11.6 mg ml^{-1} for BSA	Proteins
	Fractogel TSK AF-brown	Merck	*,b	32–63	Triazine dyes (coupled to activated Fractogel TSK HW-65)	11.6 mg ml^{-1} for BSA	Proteins
	Fractogel TSK AF-orange	Merck	*,b	32–63	Triazine dyes (coupled to activated Fractogel TSK HW-65)	11.6 mg ml^{-1} for BSA	Proteins
Cellulose	4-Aminobenzamidine coupled to succinylated aminododecylcellulose	Merck	*,b		4-Aminobenzamidine	Ligand density 90–110 μmol ml^{-1}	Proteins
	3-Aminobenzene boronic acid coupled to succinylated aminododecylcellulose	Merck	*,b		3-Aminobenzeneboronic acid	Ligand density 90–110 μmol ml^{-1}	*cis*-Diols
	Trypsin inhibitor coupled to succinylated aminododecylcellulose	Merck	*,b		Trypsin inhibitor	Ligand density 15–20 μmol ml^{-1}	Trypsin
	Trypsin (bovine) coupled to succinylated aminododecylcellulose	Merck	*,b		Trypsin	Ligand density 15–20 μmol ml^{-1}	Trypsin inhibitor

Agarose	Matrex Gel PBA	Amicon, Grace		Phenyl boronate		
	Protein A-Sepharose CL-4B	Pharmacia o,b	60–140	Protein A	Human IgG 20 mg ml^{-1}	IgG-type Antibodies, antigens, immune complexes
	Con A-Sepharose	Pharmacia o,b	60–140	Concanavalin A	Porcine thyroglobulin 8.5 mg ml^{-1}	Glycoproteins, polysaccharides, glycolipids with α-D-mannopyranose or α-D-glucopyranose
	Lentil lectin-Sepharose 4B	Pharmacia o,b	60–140	Lentil lectin	Porcine thyroglobuline 15 mg ml^{-1}	Glycoproteins with α-D-glucose or α-D-mannose
	Wheat germ lectin-Sepharose 6MB	Pharmacia o,b	45–165	Wheat germ lectin		Cells, glycoproteins and polysaccharides with N-acetylglucosamine
	Poly(U)-Sepharose 4B	Pharmacia o,b	60–140	Polyuridylic acid	m-RNA 150 μg ml^{-1}	Messenger RNA, reverse transcriptase, interferon
	Poly(A)-Sepharose 4B	Pharmacia o,b	60–140	Polyadenylic acid	2 5 S poly(U) 0 5 mg ml^{-1}	m-RNA binding proteins, poly(A) binding RNA
	Lysine-Sepharose 4B	Pharmacia o,b	60–140	L-Lysine	Plasminogen 0 6 mg ml^{-1}	Plasminogen, r-RNA, DNA
	Blue Sepharose CL-6B	Pharmacia o,b	45–165	Cibacron Blue F3G-A	Serum albumin 5 mg ml^{-1}	Enzymes requiring adenylyl-containing cofactors, albumin, interferon
	5′-AMP-Sepharose 4B			5′-AMP on C-6 spacer	LDH 10 mg ml^{-1}	NAD$^+$-dependent dehydrogenases, ATP-dependent kinases
	2′5′-ADP-Sepharose 4B			2′5′-ADP on C-6 spacer	Glucose-6-phosphate dehydrogenase 0 4 mg ml^{-1}	NADP$^+$-dependent dehydrogenases and other proteins
Cross-linked agarose	Affi-Gel Blue	Bio Rad o,b	80–150 150–130	Cibacron Blue F3GA		Enzymes, albumin, interferon
	DEAE-Affi-Gel Blue	Bio-Rad o,b		DEAE and Cibacron Blue F3GA		All serum proteins except IgG and transferrin
	CM-Affi-Gel Blue	Bio-Rad o,b		CM and Cibacron Blue F3GA		Albumin, serum protease and complement

(Continued on p 256)

TABLE 6 *(continued)*

Type	*Name*	*Supplier*	*Form*	*dp/μm*	*Activator group or ligand*	*Binding capacity*	*Ligand specifity*
	Affi-Gel 401	Bio-Rad	o,b		–SH		Sulphydryl, mercury
	Affi-Gel 501	Bio-Rad	o,b		–Hg		Sulphydryl
Polyacryl-amide	Affi-Gel 601	Bio-Rad	o,b		Phenylboronic acid	Sorbitol 130 μmole ml^{-1}	*cis*-Diol
Cross-linked agarose	Affi-Gel heparin	Bio-Rad	o,b		Heparin	Human antithrombin III 1 2 mg ml^{-1}	Various proteins
	Affi-Gel calmodulin	Bio-Rad	o,b		Calmodulin	Troporin I 0.2 mg ml^{-1}	Calcium-dependent enzymes
	Affi-Gel gelatin	Bio-Rad	o,b		Gelatin	Human plasma fibronectin 1 mg ml^{-1}	Fibronectin
	Affi-Gel Con A	Bio-Rad	o,b		Concanavalin A	Thyroglobulin 10 mg ml^{-1}	Glycoproteins, glycopeptides, saccharides
Polyacryl-amide	Affi-Gel 731	Bio-Rad	o,b		Polyethyleneimine		Membranes
Cross-linked acrylic polymer	Affi-Gel cell sorting beads, anti-mouse anti-rabbit anti-TITC	Bio-Rad	o,b,c	250 ± 50	Antibodies		B-cells/T-cells
Cross-linked agarose	Glyco Gel	Pierce	*,b	45–165	3-Aminobenzeneboronic acid		*cis*-Diols
	Detoxi Gel	Pierce	*,b	45–165			
	Detoxi Gel	Pierce	*,b	45–165			Endotoxins (lipopolysaccharides)
	Extracti-Gel D	Pierce	*,b	45–165		SDS 80 mg ml^{-1}, Triton X-100 25 mg ml^{-1}	Detergents
	Immobilized Avidin	Pierce	*,b	45–165	Avidin		Biotin-containing proteins
	Immobilized Immobiotin	Pierce	*,b	45–165	Immobiotin on spacer	Avidin 1 mg ml^{-1}	Avidin and derivatives
	Immobilized D-Biotin	Pierce	*,b	45–165	D-Biotin on spacer	Avidin 2 mg ml^{-1}	Avidin, biotin-binding proteins
	Immobilized protein A	Pierce	*,b	45–165	Protein A	Human IgG 13–15 mg ml^{-1}	Antibodies

Immobilized papain	Pierce	*,b	45–165	Loading 250 $\mu g\ ml^{-1}$	Protein digestion
Immobilized pepsin	Pierce	*,b	45–165	Loading 2–3 $mg\ ml^{-1}$	Protein digestion
Immobilized N-acetyl-D-glucosamine	Pierce	*,b	45–165	Wheat germ lectin 5–10 $mg\ ml^{-1}$	Lectins, proteins with binding specifity for that carbohydrate
Immobilized N-acetyl-D-galactosamine	Pierce	*,b	45–165	Soybean lectin 3–5 $mg\ ml^{-1}$	Lectins, proteins with binding specifity for that carbohydrate
Immobilized L-fucose	Pierce	*,b	45–165	Lotus lectin 3–5 $mg\ ml^{-1}$	Lectins, proteins with binding specifity for that carbohydrate
Immobilized D-mannose	Pierce	*,b	45–165	*Vicia fabia* lectin 3–5 $mg\ ml^{-1}$	Lectins, proteins with binding specifity for that carbohydrate
Immobilized lactose II	Pierce	*,b	45–165	Castor bean lectins 8–10 $mg\ ml^{-1}$	Lectins, proteins with binding specifity for that carbohydrate
Immobilized D-galactose	Pierce	*,b	45–165	Castor bean lectins 8–9 $mg\ ml^{-1}$	Lectins, proteins with binding specifity for that carbohydrate
Immobilized β-D-glucose	Pierce	*,b	45–165	Con A 8–10 $mg\ ml^{-1}$	Con A, proteins with β-D-Glu specifity
Immobilized N-acetyl-D,L-homo-cysteine	Pierce	*,b	45–165	Loading 23–27 $\mu mole\ ml^{-1}$ SH	Polynucleotides, enzymes
Immobilized L-alanyl-L-alanyl-L-alanine	Pierce	*,b	45–165	Elastase 2–3 $mg\ ml^{-1}$	Elastase
Immobilized *p*-aminobenzamidine	Pierce	*,b	45–165	Trypsin 7 $mg\ ml^{-1}$	Trypsin, kinases, other proteins
Immobilized *p*-amino-phenylphosphonic acid	Pierce	*,b	45–165	Calf intestinal alkaline phosphatase 0.5–1 $mg\ ml^{-1}$	Phosphatases
Immobilized (2)-Amino-ethyldihydrogen-phosphate	Pierce	*,b	45–165	Loading 33–35 $\mu mole\ ml^{-1}$	C-reactive protein

(Continued on p. 258)

TABLE 6 (*continued*)

Type	*Name*	*Supplier*	*Form*	*dp/μm*	*Activator group or ligand*	*Binding capacity*	*Ligand specifity*
	Immobilized *p*-Amino-phenylphosphoryl-choline	Pierce	*,b	45–165		Human CRP 3–5 mg ml^{-1}	C-reactive protein
	Immobilized *p*-chloro-mercuribenzoate	Pierce	*,b	45–165		Haemoglobin 40 mg ml^{-1}	Sulphydryl-containing proteins
	Immobilized Cibacron Blue F3GA	Pierce	*,b	45–165		Human serum albumin 18 mg ml^{-1}	Proteins
	Immobilized deoxycholic acid	Pierce	*,b	45–165		HSA 1 mg ml^{-1}	Membrane molecules
	Immobilized dextran sulphate	Pierce	*,b	45–165		Loading 0.5–0 6 mg ml^{-1}	Special antigens
	Immobilized diaminodipropylamine	Pierce	*,b	45–165		Loading 16–20 μmole ml^{-1}	–COOH (carbodiimide method)
	Immobilized 17-estradiol-17-hemisuccinate	Pierce	*,b	45–165			Estradiol receptors
	Immobilized fetuin	Pierce	*,b	45–165		Loading 1–2 mg ml^{-1}	Lectins
	Immobilized gelatin	Pierce	*,b	45–165		Human plasma fibro-nectin 1 mg ml^{-1}	Fibronectins
	Immobilized glycyl-L-tyrosylazobenzyl-succinic acid	Pierce	*,b	45–165		Carboxypeptidase 1 mg ml^{-1}	Carboxypeptidases
	Immobilized heparin	Pierce	*,b	45–165		Loading 0 4–0 5 mg ml^{-1}	Various proteins (*e.g.*, RNA polymerase)
	Immobilized histamine	Pierce	*,b	45–165		Loading 30–40 μmole ml^{-1}	Haeme-containing proteins,
	Immobilized imino-diacetic acid	Pierce	*,b	45–165			inorganic cation chelates bind several glyco-proteins and lipoproteins,
	Immobilized methotrexate	Pierce	*,b	45–165			dihydrofolate reductase
	Immobilized pepstatin	Pierce	*,b	45–165		Hog pepsin 1–2 mg ml^{-1}	(Acid) proteases

	Immobilized (N^{α}-CBZ)-D-phenylalanine	Pierce	*,b	45–165	Chymotrypsin 3 mg ml^{-1}	Proteases
	Immobilized poly-(L-lysine)	Pierce	*,b	45–165		Several proteins
	Immobilized Procion Red HE3B	Pierce	*,b	45–165	Human serum albumin 11–13 mg ml^{-1}	Proteins (*e.g.*, dehydrogenases)
	Immobilized protamine	Pierce	*,b	45–165	Calf thymus DNA 50 μg ml^{-1}	Several proteins
	Immobilized serotonin	Pierce	*,b	45–165	Fetuin 1 mg ml^{-1}	N-Acetylneuramic acid-containing molecules
	Immobilized spermine	Pierce	*,b	45–165	Loading 18–22 μmole ml^{-1}	Proteins, RNA
	Immobilized L-(+)-tartaric acid	Pierce	*,b	45–165	0 5–1 mg ml^{-1}	Prostatic acid, phosphatase
	Immobilized tris(carboxymethyl)-ethylenediamine	Pierce	*,b	45–165	Copper 40–45 μg ml^{-1}	Cation chelates bind proteins
	Immobilized D-tryptophan methyl ester	Pierce	*,b	45–165	α-Chymotrypsin 2–3 mg ml^{-1}	Chymotrypsin, carboxypeptidases
Cross-linked dextran	Immobilized iminodiacetic acid	Pierce	*,b	45–165		Peptides and proteins with exponated histidine or cysteine
Polyacrylamide	Immobilized lactose	Pierce	o,b	45–165		Lectins
	Immobilized melibiose	Pierce	o,b	45–165		Lectins, galactosidases
	Immobilized cellobiose	Pierce	o,b	45–165	Glucose 30–40 μmole ml^{-1}	Lectins, glucosidases
	Immobilized boronic acid	Pierce	o,b	45–165	Loading 100 μmole ml^{-1}	Nucleotides, nucleosides
Hydrophilic cross-linked vinyl polymer (Fractogel TSK HW 65 F)	Immobilized tris-(carboxymethyl)-ethylenediamine	Pierce	*,b	32–63	Copper 40–45 μg ml^{-1}	See above
	Immobilized iminodi-acetic acid	Pierce	*,b	32–63	Copper 40–45 μg ml^{-1}	See above
	Immobilized Cibacron Blue F3GA	Pierce	*,b	32–63	Human serum albumin 8–10 mg ml^{-1}	See above
	Immobilized heparin	Pierce	*,b	32–63	Loading 0 4–0 5 mg ml^{-1}	See above

(*Continued on p. 260*)

TABLE 6 *(continued)*

Type	*Name*	*Supplier*	*Form*	*dp/μm*	*Activator group or ligand*	*Binding capacity*	*Ligand specifity*
Polyacryl-amide–agarose gel	Con A-Ultrogel	IBF/LKB	*,b	100–140		Ovalbumin $\geq$0 6 mg ml^{-1}	Glc/Man
	LcA-Ultrogel	IBF/LKB	*,b	100–140		Thyroglobulin $\geq$2 mg ml^{-1}	Man/Glc
	WGA-Ultrogel	IBF/LKB	*,b	100–140		Ovomucoid $\geq$0 35 mg ml^{-1}	Glc-NAc/Di-NAc-chitobiose
	HpA-Ultrogel	IBF/LKB	*,b	100–140		A_1^+ human erythrocytes 4 5 . 10^6 ml^{-1}	Gal-NAc
	PNA-Ultrogel	IBF/LKB	*,b	100–140		Asialofetuin $\geq$0 4 mg ml^{-1}	Gal/Lact
	Protein A-Ultrogel	IBF/LKB	*,b	100–140		Human IgG 10 mg ml^{-1}	Immunoglobulins G
	Calmodulin-Ultrogel	IBF/LKB	*,b	100–140		Polylysine 0 8 mg ml^{-1}	Calcium-dependent enzymes
Cross-linked agarose	Avidin-Ultrogel A4R	IBF/LKB	*,b	60–140		Biotinylated albumin 1 mg ml^{-1}	Biotin
	Gelatin-Ultrogel	IBF/LKB	*,b	60–140		Human fibronectin 1 mg ml^{-1}	Cell adhesion factors
	ss DNA-Ultrogel A4R	IBF/LKB	*,b	60–140		Protamine sulphate 5–10 mg ml^{-1}	DNA-dependent or -containing proteins
	Acriflavine-Ultrogel A4R	IBF/LKB	*,b	60–140		Loading 15–20 μmole ml^{-1}	Aromatic molecules (nucleic acids, dyes)
	Phospho-Ultrogel A6R	IBF/LKB	*,b	60–140		Loading 100 μmole ml^{-1} acidic groups	Phosphatases
	Blue-Ultrogel A4R	IBF/LKB	*,b	60–140		Human albumin 12 mg ml^{-1}	Various proteins
	Heparin-Ultrogel A4R	IBF/LKB	*,b	60–140		Human antithrombin III 1 8–2 8 mg ml^{-1}	Various proteins
Acrylic polymer	Oligo$(dT)_8$-Trisacryl M	IBF/LKB	*,b	60–140		Poly(A) 0.15 mg ml^{-1}	Eucaryontic m-RNA
	Blue-Trisacryl M	IBF/LKB	*,b	40–80		Human albumin 10–15 mg ml^{-1}	Various proteins

does not provide sufficient purity and thus a sequence of modes is applied in the appropriate manner.

Resolution apart, LC phase systems have very different loadability characteristics. Again, therefore, the selection of an LC phase system is dictated by the maximization of the chromatographic resolution times the maximum sample input, as shown in eqn. 6.

The characteristics of LC phase systems, together with other relevant aspects, are briefly discussed below.

6.1. Size exclusion chromatography (SEC)[23,24]

SEC has the lowest resolution of all LC modes, which can be attributed to the limited separation volume of SEC columns. Hence, SEC is suitable for the fractionation of sample mixtures according to molecular size and shape of solutes, and is the method of choice for desalting. It should be noted that SEC columns cannot be loaded with large feed volumes. The maximum feed volume while maintaining maximum resolution under analytical conditions is about 1–2% of the total column volume[24]. One advantage of SEC is that the solutes are eluted in a short and predictable time interval.

6.2. Adsorption chromatography

Both normal- and reversed-phase chromatography (RPC) are methods for achieving high resolution. In comparison with RPC, normal-phase chromatography with native silica or other adsorbents offers several advantages, *e.g.*, reasonably priced packings, application of a broad range of solvents as eluents, easy removal of solvents by evaporation after fractionation, high solubility of sample mixture components, application of high flow-rates owing to the low viscosity of the eluents and analysis of fractions by thin-layer chromatography. Drawbacks are the relatively long calibration periods of normal-phase systems[20] and surface fouling by strong, irreversible adsorption of polar contaminants. The latter can be prevented by employing a pre-column or by using adsorbents with moderate specific surface areas (a_s ~ 100 m^2 g^{-1}).

RPC is universally applicable to polar, hydrophobic and ionized solutes. Regeneration and equilibration of phase systems is faster than for plain silica. The use of aqueous or aqueous–organic eluents causes higher column pressure drops under comparable conditions than do the low-viscosity eluents in normal-phase chromatography, making the isolation of purified products more tedious.

Reversed-phase gradient LC can also be applied for the isolation of peptides and polypeptides in acidic hydro–organic eluents of low ionic strength[25]. The solutes are separated according to their hydrophobic or hydrophilic character. Problems often arise in the maintenance of the biological activity of biopolymers.

Hydrophobic interaction chromatography is a more suitable method of protein isolation with regard to the preservation of biological activity, applying a descending salt gradient at neutral pH[26].

6.3. *Ion-exchange chromatography (IEC)*

IEC offers a high selectivity and resolution when a pH or salt gradient is applied. It has been found particularly efficient in the isolation of proteins[27]. Ion-exchange columns have a high loadability and are preferentially employed during the initial purification stages when large amounts of sample mixtures are handled. The loadability is of the order of 100 mg/g of ion exchanger and is dependent on the surface area of the packing.

6.4. *Affinity chromatography (AC)*[28]

Compared with the other LC modes, AC possesses the highest selectivity and flexibility towards biospecific solutes. For example, immunoadsorbents with coupled monoclonal antibodies have an exceptionally high biospecific recognition[29]. The flexibility is based on the availability of activated affinity media for the purpose of binding specific ligands; the specificivity can be further controlled by the elution conditions.

7 FUTURE PROSPECTS

With a few exceptions, the tailor-made manufacture of packings for preparative and process chromatography has as yet received very little attention. This situation, however, is bound to change radically in the near future as a result of the dramatic increase in chromatographic separation methods in the areas of biotechnology and gene technology. Together with the improvements in biotechnological processes, research and development in packings will focus on three major topics:

(i) the synthesis of better defined, more reproducible and biocompatible supports that meet the required criteria,

(ii) the bonding chemistry in the synthesis of a limited number of well characterized and stable packings that offer high flexibility by further modification to multi-mode chromatography; and

(iii) the synthesis of highly biospecific packings for the selective recognition and isolation of biologically active solutes

8 LIST OF SUPPLIERS

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Bio-Rad Laboratories, 2200 Wright Avenue, Richmond, CA 94804, U S.A.
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9 SUMMARY

Although the theoretical treatment of chromatographic processes on a preparative scale provides guidelines to the extent to which packing and stationary phase properties affect the target quantities such as sample input, throughput and resolution times sample input, a series of additional criteria were established to judge the quality of a packing in preparative column liquid chromatography. These include bed stability and flow resistance, chemical resistance and purity, solute accessibility, mass and biological recovery, fouling, regeneration and cost. Applying these criteria, the relative importance of physical and chemical structure parameters of packings and stationary phases was assessed. Commercial packings with mean particle diameters $d_p > 20$ μm were listed for adsorption, size exclusion, ion-exchange and affinity chromatography. An analysis of the characteristic features of phase systems showed that adsorption media offer a high selectivity combined with adequate loadability, whereas ion exchangers and affinity media were best suited for biospecific solutes, particularly biopolymers, which can be attributed to their high selectivity and loadability.

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