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Performance comparison of low-pressure ion-exchange chromatography media for protein separation

Peter R. Levison*, Carol Mumford, Michael Streater, Anne Brandt-Nielsen, Navin D. Pathirana, Stephen E. Badger

Whatman International Ltd., Springfield Mill, James Whatman Way, Maidstone, Kent, ME14 2LE, UK

Abstract

The physical and functional performance of 70 different anion- and cation-exchange media based on cellulose, agarose, dextran and polymeric/composite materials has been evaluated. Physical tests such as swelling, flow performance and column packing densities and functional tests including small-ion capacity, protein capacity and chromatographic performance were carried out on each grade. The data, descriptive rather than prescriptive, demonstrates significant performance differences from medium to medium and suggest that a rigorous media screening exercise be carried out prior to developing an ion-exchange separation process, in order to optimise the efficiency of the process.

Keywords: Stationary phase, LC; Proteins

1. Introduction

Ion-exchange chromatography is routinely used in the downstream processing of commercially important bioproducts. Proteins are based on copolymers of amino acids [1] and thus may be regarded as polyions. At a given pH they will bear either a positive or negative charge dependent on their isoelectric point, p*I*, which is influenced by their primary, secondary, tertiary and quaternary structures as well as structural elements such as glycosylation arising from post-translational modification. Protein purification by ion-exchange utilises anion or cation exchangers, functionalised with amines or acids, respectively [2] and traditionally attached to polysaccharide matrices including cellulose, agarose and dextran [3]. More recently ion exchangers based on composite polymers have been introduced [4]. Protein separations can be carried out in either a positive or negative ion-exchange step, where either the target or contaminants are retained, respectively [5]. Biochemical applications of process-scale ion-exchange liquid chromatography include the isolation of uridine phosphorylase from *Escherichia coli* [6], prochymosin from *Escherichia coli* [7], L-asparaginase from *Erwinia* spp. [8], Monoclonal antibodies [5,9], albumin from human plasma [10], proteins from hen egg-white [11,12], immunoglobulin G from goat serum [13] and DNA modifying enzymes from microbial sources [14].

When establishing an ion-exchange protocol the protein chromatographer is faced with a myriad of related adsorbents available from different vendors each bearing similar functional groups but attached to different matrices using proprietary chemical techniques. In the field of affinity chromatography there have been several small studies comparing characteristics of the base matrix and how it may affect performance in the affinity process [15–18]. However for ion exchange the influence of the base

*Corresponding author.

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matrix has not been widely reported. If a chromatographer is considering a large-scale batch process then a matrix which has low shear sensitivity would be preferred [19,20]. In column processes, resistance to bed collapse when pressure is applied is important in order to maintain flow [20,21], although this could to a degree be offset if one was to switch from an axial flow to a radial flow column [22]. More recently macroporous particles have been developed facilitating use at very high flow-rates, since diffusional limitations apparent in traditional ion exchangers were reduced [23,24], although their use at large-scale presupposes appropriate hardware is available to support such flow-rates.

While there are a plethora of publications on ion-exchange protein separations, there has been little attention to screening various ion exchangers for the same separation. We reported a limited comparison of some cellulose and agarose ion exchangers [2,25] and more recently have carried out a detailed evaluation into the process-scale purification of hen egg-white proteins on the anion-exchange cellulose Whatman Express-Ion Exchanger Q and the agarose Pharmacia Q-Sepharose Fast Flow [26].

In the present study we report a comparative screen of various commercially available ion exchangers obtained from various vendors. The data is descriptive rather than prescriptive and highlights many differences in the biochemical application of these products.

2. Experimental

2.1. Materials

Ion-exchange media were obtained as follows: DE51, DE52, DE53, QA52, CM52, SE52, SE53, Express-Ion D, Express-Ion Q, Express-Ion C and Express-Ion S were obtained from Whatman International (Maidstone, UK). DEAE-Sephacel, DEAE-Sepharose CL6B, DEAE-Sepharose Fast Flow, Q-Sepharose Fast Flow, Q-Sepharose HP, CM-Sepharose Fast Flow, S-Sepharose Fast Flow, S- Sepharose HP, DEAE-Sephadex A-25, DEAE-Sephadex A-50, QAE-Sephadex A-25, QAE-Sephadex A-50, CM-Sephadex C-25, CM-Sephadex C-50, SP-Sephadex C-25 and SP-Sephadex C-50 were obtained from Pharmacia Biotech (St Albans, UK). Matrex DEAE A-200 Cellufine, Matrex DEAE A-500 Cellufine, Matrex DEAE A-800 Cellufine, Matrex CM C-200 Cellufine and Matrex CM C-500 Cellufine were obtained from Amicon (Stonehouse, UK). DEAE Thruput, CM Thruput and Q Thruput were obtained from Sterogene (Carlsbad, USA). DEAE Toyopearl 650 S, DEAE Toyopearl 650 M, DEAE Toyopearl 650 C, CM Toyopearl 650 S, CM Toyopearl 650 M, CM Toyopearl 650 C, SP Toyopearl 550 C, SP Toyopearl 650 S, SP Toyopearl 650 M and SP Toyopearl 650 C were obtained from Toso Haas (Tokyo, Japan). Macro Prep Q, Macro Prep High Q, Macro Prep CM, Macro Prep S and Macro Prep High S were obtained from Bio-Rad (Hemel Hempstead, UK). Fractogel EMD TMAE-650, Fractogel EMD DEAE-650, Fractogel EMD DMAE- 650 and Fractogel EMD SO_3^--650 were obtained from Merck (Poole, UK). Poros 50 HQ and Poros 50 HS were obtained from PerSeptive Biosystems (Freiburg, Germany). DEAE-Trisacryl M, DEAE-Trisacryl Plus M, CM-Trisacryl M, SP-Trisacryl M, SP-Trisacryl Plus M, DEAE-Spherodex M, CM- Spherodex M, SP-Spherodex M, DEA-Spherosil M, QMA-Spherosil M, DEAE HyperD F, Q HyperD M and S HyperD M were obtained from BioSepra (Boreham-wood, UK).

2.2. Methods

Each ion-exchange medium was handled according to the media manufacturers instructions. The media were each cycled in either 0.5 *M* HCl then 0.5 *M* NaOH for anion exchangers, or 0.5 *M* NaOH then 0.5 *M* HCl for cation exchangers. In the case of Fractogel 0.2 *M* NaOH and 0.2 *M* HCl were used. Each precycled medium was collected by filtration and used for subsequent testing. Each medium was tested for regains, small-ion capacity, protein binding capacity (bovine serum albumin for anion exchangers and lysozyme for cation exchangers) and desorption efficiency, column flow-rate and chromatographic performance. The testing was carried out according to standard quality control (QC) test methods used by Whatman International in accord with their ISO9001 accreditation.

3. Results and discussion

Quality control testing is a crucial step in ion-exchange media manufacture and it is used in ensuring that individual batches of medium meet certain criteria necessary for effective use in their chromatographic application. Where companies have an external quality accreditation typically ISO9001 then quality standards and test protocols etc., are laid down as controlled documents in their operating procedure manuals. When an end-user purchases ion-exchange media for a process application he would typically perform some incoming raw material QC using either vendor supplied protocols or in-house variants thereof. In this context we determined to compare a range of some 70 commercially available ion-exchange chromatography media by testing them under identical conditions using a standard test regime. The rationale behind the testing was as follows:

3.1. Regains

Regains are a measure of the swelling of the ion-exchange particles and are essentially a loss on drying expressed as grams of imbibed moisture/gram dry exchanger. Regains are determined in both ionised and deionised forms and any differences in regain values between these two values indicates the effect of pH on dimensional stability of the particle,

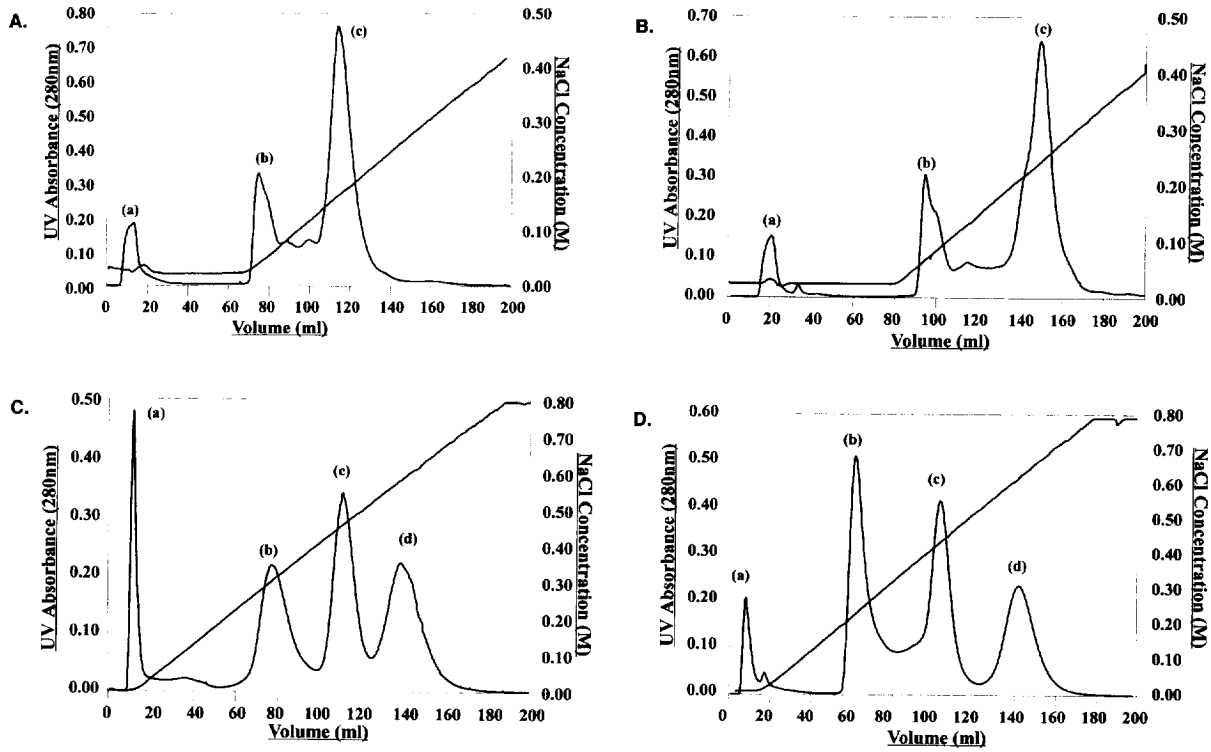

Fig. 1. Chromatographic performance testing of (A) DE52 in a column (10 cm×1.5 cm I.D.) in 0.025 *M* Tris–HCl buffer, pH 7.5 containing 0–0.5 *M* NaCl at a flow-rate of 2.0 ml/min. Test mixture (7 ml) containing 100 mg natural hen egg-white. Eluting peaks correspond to (a) lysozyme, (b) conalbumin and (c) ovalbumin; (B) QA52 in a column (15 cm×1.5 cm I.D.) all other details as per DE52; (C) CM52 in a column (10 cm×1.5 cm I.D.) in 0.01 *M* sodium acetate buffer, pH 4.5 containing 0–0.8 *M* NaCl at a flow-rate of 2.0 ml/min. Test mixture (1.5 ml) containing ATP (1 mg), ovalbumin (30 mg), cytochrome *c* (14 mg) and lysozyme (8 mg). Eluting peaks correspond to (a) ATP, (b) ovalbumin, (c) cytochrome *c* and (d) lysozyme; (D) SE52 in a column (10 cm×1.5 cm I.D.) in 0.01 *M* sodium acetate buffer, pH 4.8 containing 0–0.8 *M* NaCl at a flow-rate of 2.0 ml/min. Test mixture (1.5 ml) containing ATP (1 mg), conalbumin (30 mg), cytochrome *c* (15 mg) and lysozyme (8 mg). Eluting peaks correspond to (a) ATP, (b) conalbumin, (c) cytochrome *c* and (d) lysozyme.

a factor of importance when considering scaling-up a column process requiring periodic clean in place [26].

3.2. Small-ion capacity

Small-ion capacity expressed as mequivalents/dry gram exchanger is a measure of the number of ionizable groups on the exchanger [27]. This value obtained by titration may be expressed as mmoles for a monovalent ion where mequivalents equal mmoles.

3.3. Protein capacity

Protein capacity expressed both in terms of mg protein/dry gram exchanger (to eliminate swelling/regain changes) and mg/ml column volume indicates the practicality of the ion exchanger [27]. However, as we have described previously the protein capacity of an ion exchanger is variable dependent on molecular mass and pH [28]. A desorption test is carried out whereby the protein is only partially desorbed from the exchanger. This gives an indication of binding strength attributed presumably to the number of ionic groups on the matrix interacting with charged regions of the protein. This influences chromatographic resolution and thus selectivity of the medium [29].

3.4. Column packing density

Column packing density is used to convert values determined in terms of dry mass of exchanger to column volume equivalents, the working values. This value will however be influenced by mobile phase composition, regain values and column packing protocols [20,21].

3.5. Flow-rate

Flow-rate was determined at two standard pressures 50 cmH_2O/cm and 75 cmH_2O/cm, where 1 cmH_2O=98.0665 Pa, using a laboratory column. While this is a useful comparative test, it should be noted that linear flow-rates reduce ca. 5-fold when a process is scaled-up to a process column (i.e., 45 cm I.D.) due to wall effects [26].

3.6. Chromatographic performance testing

Chromatographic performance testing gives an indication that the ion exchanger is fit for purpose. In each case we use a multi component mixture for test chromatography. Typical test chromatograms for DE52, QA52, CM52 and SE52 are shown in Fig. 1. Hen egg-white is a good model system for anion exchangers when used at pH 7.5 [26] and the test protein mixtures are suitable for cation exchangers. In order to compare the chromatographic performance of each medium a degree of resolution may be calculated according to:

$$\text{Degree of resolution} = \frac{(V_2 - V_1)}{(W_2 + W_1)} \cdot 2$$

where for anion exchangers V_2 = elution volume of ovalbumin, V_1 = elution volume of conalbumin, W_2 = peak width of ovalbumin at 0.5 peak height and W_1 = peak width of conalbumin at 0.5 peak height. In the case of the cation exchangers V_1 and W_1 relate to either ovalbumin or conalbumin for C or S exchangers respectively. The higher the degree of resolution the better the selectivity and peak shape.

The test data obtained for the cellulose-based ion exchangers are summarised in Table 1, data for agarose-based ion exchangers in Table 2, data for dextran-based ion exchangers in Table 3 and data for polymeric/composite ion exchangers in Table 4. Looking at the data for the polysaccharide-based exchangers (Tables 1–3) illustrates the differences between ion exchangers bearing similar chemistries on similar matrices, but also the differences as one goes from one matrix to another. The test data for polymeric/composite matrices (Table 4) is very wide, perhaps not surprising due to the number of different base matrices used in their fabrication.

For reasons discussed earlier, factors such as dimensional stability (difference in regains) or flow performance may direct the chromatographer to select certain media in preference to others, and personal preferences based on previous experiences may come to bear. However, it is quite apparent from the data on degree of resolution, that the chromatographic performance of each medium varied quite significantly from the next. It is this aspect where media selection is critical. In this study we

Table 1
Test data on cellulose-based ion exchangers

Media grade	Regains (g/dry g)		Small-ion capacity (mequiv./dry g)	Protein capacity[a] (mg/dry g)		Column packing density (dry g/ml)	Protein capacity (mg/ml)	Flow-rate (cm/h)		Degree of resolution
	Ionised	Deionised		absorp.	% desorp.			50 cmH_2O/cm	75 cmH_2O/cm	
DE51	2.53	2.46	0.25	152	72.0	0.17	26	168	234	–
DE52	3.05	2.55	0.98	700	37.6	0.18	126	155	221	3.75
DE53	2.70	2.84	2.00	640	18.1	0.18	115	94	137	5.33
Express-Ion D	1.92	1.88	0.98	290	28.4	0.23	67	425	614	2.88
DEAE-Sephacel	5.40	5.13	1.52	1110	24.9	0.10	111	166	205	2.07
DEAE A-200 Cellufine	3.80	2.75	1.03	930	37.9	0.16	149	387	556	2.00
DEAE A-500 Cellufine	4.24	4.55	1.34	630	36.4	0.12	76	519	759	2.36
DEAE A-800 Cellufine	5.25	4.97	0.97	740	42.0	0.10	74	238	316	2.08
QA52	2.72	2.64	1.20	730	32.9	0.22	161	77	259	4.67
Express-Ion Q	1.78	1.82	0.89	350	31.9	0.25	87	477	696	3.50
CM52	3.50	2.42	1.02	1150	–	0.15	173	147	210	2.53
Express-Ion C	1.97	1.78	0.85	660	–	0.24	158	455	643	2.70
CM C-200 Cellufine	4.95	4.71	1.19	700	–	0.13	91	152	187	3.12
CM-C-500 Cellufine	3.48	2.48	0.62	890	–	0.17	151	705	984	1.36
SE52	3.31	–	0.89	1270	65.9	0.18	229	98	127	4.57
SE53	3.26	–	2.25	1230	9.5	0.19	234	148	212	7.83
Express-Ion S	2.28	–	0.92	640	54.3	0.22	141	378	530	4.80

[a] Bovine serum albumin—anion exchangers; lysozyme—cation exchangers.

Table 2
Test data on agarose-based ion exchangers

Media grade	Regains (g/dry g)		Small-ion capacity (mequiv./dry g)	Protein capacity[a] (mg/dry g)		Column packing density (dry g/ml)	Protein capacity (mg/ml)	Flow-rate (cm/h)		Degree of resolution
	Ionised	Deionised		Absorp.	% Desorp.			50 cmH_2O/cm	75cmH_2O/cm	
DEAE-Sepharose CL-6B	7.33	7.40	2.22	1120	6.3	0.07	78	90	112	3.56
DEAE-Sepharose Fast Flow	7.15	8.10	1.89	602	35.6	0.08	48	470	677	2.90
DEAE Thruput	7.78	6.90	2.02	1100	5.2	0.08	88	513	726	1.49
Q-Sepharose Fast Flow	4.67	4.86	1.45	648	33.9	0.12	78	579	835	4.00
Q-Sepharose HP	5.89	6.20	1.65	770	26.9	0.09	69	102	141	3.53
Q Thruput	8.79	8.15	0.39	690	70.9	0.06	41	668	837	ND[b]
CM Sepharose Fast Flow	8.63	8.57	1.74	1520	–	0.07	106	408	585	3.59
CM Thruput	10.50	8.38	1.91	990	–	0.06	59	439	596	2.22
S Sepharose Fast Flow	4.45	–	1.40	1095	64.7	0.12	131	499	718	3.82
SP Sepharose HP	5.04	–	1.47	710	66.6	0.09	64	121	166	6.40

[a] Bovine serum albumin—anion exchangers; lysozyme—cation exchangers.
[b] ND—no resolution demonstrated under these chromatographic conditions.

Table 3
Test data on dextran-based ion exchangers

Media grade	Regains (g/dry g)		Small-ion capacity (mequiv./dry g)	Protein capacity (mg/dry g)		Column packing density (dry g/ml)	Protein capacity (mg/ml)	Flow-rate (cm/h)		Degree of resolution
	Ionised	Deionised		Absorp.	% Desorp.			50 cmH_2O/cm	75 cmH_2O/cm	
DEAE-Sephadex A-25	3.78	3.34	3.36	570	16.3	0.17	97	544	775	2.80
DEAE-Sephadex A-50	≥10	≥10	3.48	1080	3.5	0.03	32	43	46	ND[b]
QAE Sephadex A-25	3.26	4.58	2.44	280	4.8	0.17	48	179	282	2.54
QAE Sephadex A-50	≥10	≥10	2.80	1040	3.4	0.04	42	61	62	1.86
CM Sephadex C-25	5.25	1.59	4.35	900	–	0.12	108	462	642	1.52
CM Sephadex C-50	≥10	8.71	4.76	860	–	0.02	17	234	237	ND[b]
SP Sephadex C-25	4.26	–	1.87	1722	27.6	0.15	259	571	788	2.00
SP Sephadex C-50	≥10	–	1.39	1870	43.9	0.02	37	236	238	1.16

[a] Bovine serum albumin—anion exchangers; lysozyme—cation exchangers.
[b] ND—no resolution demonstrated under these chromatographic conditions.

Table 4
Test data on polymer/composite-based ion exchangers

Media grade	Regains (g/ dry g)		Small-ion capacity (mequiv./dry g)	Protein capacity[a] (mg/dry g)		Column packing density (dry g/ml)	Protein capacity (mg/ml)	Flow-rate (cm/h)		Degree of resolution
	Ionised	Deionised		Absorp.	%Desorp.			50 cmH_2O/cm	75 cmH_2O/cm	
Fractogel EMD TMAE-650	2.31	2.37	0.28	410	38.6	0.19	78	509	703	2.11
Fractogel EMD DEAE-650	2.32	2.26	0.50	390	47.6	0.21	82	445	621	3.50
Fractogel EMD DMAE-650	2.36	2.26	0.56	200	86.4	0.21	42	460	653	4.00
DEAE-Toyopearl 650S	2.13	2.28	0.69	179	37.9	0.19	34	252	360	1.93
DEAE-Toyopearl 650M	2.12	2.27	0.63	196	32.8	0.20	39	716	1014	4.00
DEAE-Toyopearl 650C	1.91	2.04	0.60	188	33.5	0.21	39	1073	1491	1.57
DEAE-Trisacryl M	2.44	1.95	1.67	70	83.9	0.25	18	115	150	ND[b]
DEAE-Trisacryl Plus M	3.35	2.71	1.94	590	45.8	0.18	106	75	108	2.73
DEAE-Spherodex M	0.98	0.96	0.27	340	56.3	0.46	156	520	727	3.80
DEA-Spherosil M	0.49	0.26	0.07	50	56.3	0.39	19	681	982	ND
DEAE Hyper D F	0.80	0.81	0.33	320	48.7	0.50	160	245	348	3.12
QAE-Toyopearl 550 C	3.34	3.73	3.01	485	46.2	0.16	78	379	545	3.47
Macro Prep Q	1.55	1.61	0.99	120	64.8	0.25	30	301	422	0.80
Macro Prep High Q	1.83	2.14	1.88	210	23.8	0.21	44	462	656	3.22
QMA-Spherosil M	0.92	0.92	0.15	150	14.3	0.44	66	577	793	ND
Q Hyper D M	0.78	0.79	0.21	240	17.6	0.46	110	729	994	4.60
Poros 50 HQ	1.66	1.63	0.64	80	94.9	0.24	19	340	505	4.10
CM-Toyopearl 650 S	2.16	2.08	0.46	228	–	0.21	48	222	320	5.00
CM-Toyopearl 650 M	1.99	1.91	0.50	198	–	0.22	44	396	565	4.28
CM-Toyopearl 650 C	1.92	1.83	0.46	286	–	0.22	63	907	1302	3.50
Macro Prep CM	1.44	1.18	0.72	100	–	0.31	31	246	341	1.89
CM-Trisacryl M	2.47	1.53	0.76	870	–	0.24	209	111	143	3.20
CM-Spherodex M	1.00	0.96	0.21	120	–	0.39	47	800	1103	3.24
Fractogel EMD SO_3^--650	2.47	–	0.66	560	39.4	0.21	118	347	487	4.29
SP-Toyopearl 550 C	2.18	–	0.76	628	36.5	0.20	126	700	1006	2.44
SP-Toyopearl 650 S	2.07	–	0.76	198	100	0.22	44	228	330	5.50
SP-Toyopearl 650 M	2.01	–	0.80	224	99.1	0.21	47	665	959	3.14
SP-Toyopearl 650 C	2.02	–	0.81	274	86.1	0.21	58	1131	1628	2.54
Macro Prep S	1.21	–	0.58	470	21.6	0.31	145	383	533	ND
Macro Prep High S	1.21	–	0.44	330	19.0	0.32	106	473	651	ND
SP-Trisacryl M	3.14	–	1.19	1010	60.2	0.19	192	156	191	2.12
SP-Trisacryl Plus M	3.19	–	1.36	1250	43.8	0.11	137	150	196	3.50
SP-Spherodex M	1.61	–	0.20	320	18.5	0.42	134	752	1033	ND
S Hyper D M	0.77	–	0.23	250	78.4	0.44	110	972	1308	2.36
Poros 50 HS	1.18	–	0.41	220	62.6	0.32	70	147	209	4.50

[a] Bovine serum albumin—anion exchangers; lysozyme—cation exchangers.

[b] ND—No resolution demonstrated under these chromatographic conditions.

have not optimised the chromatographic conditions for particular grades of ion exchanger simply substituted one for another in a standard test. This approach while suitable for rapid screening, runs a serious risk of giving misleading data and could result in a chromatographer discarding an ion exchanger, which if the conditions were optimised could in fact out perform other grades.

The purpose of this work was not to be prescriptive in terms of recommending particular ion-exchange media. Rather the data presented here, clearly demonstrates that when comparing some 70 different anion and cation-exchange media there are significant performance differences between them. These differences are manifest both when different manufacturers prepare similar chemistries on similar matrices, i.e., cellulose or agarose and also when similar chemistries are prepared on differing matrices i.e., dextran versus composite polymers.

When developing an ion-exchange process, simply using the media which worked last time may not be the best approach. Due to the variability of commercially available ion exchangers for a common set of performance tests, both physical and functional, it is recommended that protein chromatographers rigorously screen media prior to scale-up in order to optimise process throughput and the commercial viability of the purification.

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