

(12) PATENT
(19) AUSTRALIAN PATENT OFFICE

(11) Application No. **AU 200053817 B2**
(10) Patent No. **778045**

(54) Title
Process for making fluorinated polymer adsorbent particles

(51) ⁶ International Patent Classification(s)
C08J 009/20 C08J 009/28
B01D 015/08 C08L 033/00
B01J 020/26

(21) Application No: 200053817 (22) Application Date: 2000.06.12

(87) WIPO No: W000/77081

(30) Priority Data

(31) Number	(32) Date	(33) Country
09/329289	1999.06.10	US

(43) Publication Date : 2001.01.02
(43) Publication Journal Date : 2001.03.15
(44) Accepted Journal Date : 2004.11.11

(71) Applicant(s)
Prometic Biosciences Inc.

(72) Inventor(s)
Leonard H. Smiley; Christopher Lowe; Julie Tucker

(74) Agent/Attorney
Davies Collison Cave, Level 15, 1 Nicholson Street, MELBOURNE VIC 3000

(56) Related Art
US 4171412
US 4035316
US 3985632

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau(43) International Publication Date
21 December 2000 (21.12.2000)

PCT

(10) International Publication Number
WO 00/77081 A1(51) International Patent Classification: C08J 9/20, B01J
20/26, B01D 15/08, C08J 9/28 // C08L 33/00(74) Agents: LOOPER, Ywe et al.: Smart & Biggar, 900-55
Metcalf Street, P.O. Box 2999, Station D, Ottawa, Ontario
K1P 5Y6 (CA).

(21) International Application Number: PCT/CA00/00701

(22) International Filing Date: 12 June 2000 (12.06.2000)

(25) Filing Language: English

(26) Publication Language: English

(81) Designated States (national): AE, AG, AL, AM, AT, AU,
AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE,
DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU,
ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS,
LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ,
PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT,
TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.(84) Designated States (regional): ARIPO patent (GH, GM,
KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian
patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European
patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE,
IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG,
CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).(30) Priority Data:
09/329,289 10 June 1999 (10.06.1999) US(71) Applicant (for all designated States except US):
PROMETIC BIOSCIENCES INC. [CA/CA]; 6100
Royalmount, Montreal, Quebec H4P 2R2 (CA).

Published:

- With international search report.
- Before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments.

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.



(72) Inventors; and

(75) Inventors/Applicants (for US only): SMILEY, Leonard,
H. [US/US]; 1624 Waverly Street, Philadelphia, PA 19146
(US). BIGWOOD, Michael [US/US]; 513 Wisconsin
Avenue, Orland, PA 19075 (US). LOWE, Christopher
[GB/GB]; The Limes, Hempstead, Saffron Walden,
Cambs CB10 2PW (GB). TUCKER, Julie [GB/GB]; 17
Henswood Crescent, Pembury, Kent TN2 4LJ (GB).

WO 00/77081 A1

(54) Title: PROCESS FOR MAKING FLUORINATED POLYMER ADSORBENT PARTICLES

(57) Abstract: A process to make particles by anaerobic reaction of a water-insoluble solution of organic compounds comprising (a) a monomer selected from C₂₋₄ alkylene glycol esters of a C₃₋₆ acrylic acid and a divinyl benzene; (b) a polyfluorinated vinyl monomer; (c) a monomer selected from acrylic acid, methacrylic acid and esters thereof; (d) a free radical initiator; and (e) a water-insoluble, organic solvent-soluble porogenic material, the weight ratio of comonomers (a) plus (b) plus (c) to the porogenic material being from 0.5:1 to 2:1. The adsorbent particles produced by the process are useful in carrying out chromatographic separations, or in the production of medical devices.

PROCESS FOR MAKING FLUORINATED POLYMER ADSORBENT PARTICLES**FIELD OF INVENTION**

The invention relates to a process for making
5 fluorinated polymer adsorbent particles and to their use as a
stationary phase for carrying out chromatographic separations.

BACKGROUND OF THE INVENTION

Support materials for use in high productivity liquid
chromatography must be mechanically strong in order to
10 withstand operation at high rates of flow under high pressures.
Moreover, they must be stable over the wide range of pH to
which such materials are subjected during normal operation and
regeneration. The stability of the polymeric particles in its
environment allows it to withstand degradation and
15 decomposition. Physical properties of particular importance to
chromatographic media are (1) sphericity of the particles; (2)
high surface area; (3) high pore volume and availability; (4)
wide range of pore diameters; and (5) wide range of particle
diameters.

20 The particles of the invention are an improvement
over known particles in respect of many of the above
properties. Furthermore, the fluorinated surface of certain of
the particles of the invention present unusual and unexpected
polarity that is beneficial in performing chromatographic
25 separations such as that used for DNA.

SUMMARY OF THE INVENTION

The invention is therefore directed to the
manufacture of improved fluorinated particles having adsorbent
properties for superior performance as the stationary phase for
30 use in chromatographic separations.

SUBSTITUTE SHEET (RULE 26)

The invention provides a process for the preparation of porous spherical particles of fluorinated polymer adsorbent comprising the steps of:

(1) forming a water-insoluble solution of organic
5 compounds comprising a monomer selected from C₂₋₄ alkylene glycol esters of a C₃₋₆ acrylic acid or divinyl benzene; a polyfluorinated vinyl monomer; a free radical initiator; and a water-insoluble, organic solvent-soluble porogenic material, the weight ratio of the comonomers to porogenic material being
10 from 0.5:1 to 2:1;

(2) forming a dilute solution of a dispersing agent in water from which any oxygen has been purged with inert gas;

(3) with agitation and inert gas purging, rapidly dispersing the water-insoluble solution of organic compounds
15 from step (1) into the dilute aqueous solution from step (2) and, as necessary, adjusting the temperature of the dispersion to 30-90°C to initiate copolymerization of the monomers, the level of mixing energy being sufficient to disperse the water-insoluble solution of organic compounds in the solution from
20 step (2) in the form of liquid droplets having an average diameter of no more than 10-300 micrometers, at least 90% of the droplets being within 40% above or below the average mean particle diameter;

(4) continuing the agitation and oxygen purging of
25 the dispersion from step (3) for a time sufficient to effect complete copolymerization of the monomers and particulation of the droplets in the form of finely divided polymer particles by precipitation of the copolymer therein;

(5) separating the finely divided copolymer particles
30 from the polymerization reaction medium;

SUBSTITUTE SHEET (RULE 26)

(6) extracting the porogenic material from the separated copolymer particles of step (5) by washing the particles with inert organic solvent, thereby forming pores within the copolymer; and

5 (7) drying the porous copolymer particles.

The invention further provides a process for the preparation of porous spherical particles of fluorinated polymer adsorbent comprising the steps:

(1) forming a water-insoluble solution of organic
10 compounds comprising (a) a monomer selected from C_{2-4} alkylene glycol esters of a C_{3-6} acrylic acid and a divinyl benzene; (b) a polyfluorinated vinyl monomer; (c) a monomer selected from acrylic acid, methacrylic acid and esters thereof; (d) a free
15 radical initiator; and (e) a water-insoluble, organic solvent-soluble porogenic material, the weight ratio of comonomers (a) plus (b) plus (c) to the porogenic material being from 0.5:1 to 2:1;

(2) forming a dilute solution of a dispersing agent in water from which any oxygen has been purged with inert gas;

20 (3) with agitation and inert gas purging rapidly dispersing the water-insoluble solution of organic compounds from step (1) into the dilute aqueous solution from step (2) and, as necessary, adjusting the temperature of the dispersion to 30-90°C to initiate copolymerization of the monomers, the
25 level of mixing energy being sufficient to disperse the water-insoluble solution of organic compounds in the solution from step (2) in the form of liquid droplets having an average diameter of no more than 10-300 micrometers, at least 90% of the droplets being within 40% above or below the average mean
30 particle diameter;

SUBSTITUTE SHEET (RULE 26)

- 4 -

(4) continuing the agitation and oxygen purging of the dispersion from step (3) for a time sufficient to effect complete copolymerization of the monomers and particulation of the droplets in the form of finely divided polymer particles by precipitation of the copolymer therein;

(5) separating the finely divided copolymer particles from the polymerization reaction medium;

(6) extracting the porogenic material from the separated copolymer particles of step (5) by washing the particles with inert organic solvent, thereby forming pores within the copolymer; and

(7) drying the porous copolymer particles.

The invention further provides adsorbent particles made by the process described above.

The invention further provides a water-insoluble solution of organic compounds comprising (a) a monomer selected from C_{2-4} alkylene glycol esters of a C_{3-6} acrylic acid and a divinyl benzene; (b) a polyfluorinated vinyl monomer; (c) a monomer selected from acrylic acid, methacrylic acid and esters thereof; (d) a free radical initiator; and (e) a water-insoluble, organic solvent-soluble porogenic material, the weight ratio of comonomers (a) plus (b) plus (c) to the porogenic material being from 0.5:1 to 2:1.

The present invention further provides uses for the particles according to the invention as a stationary phase in chromatographic techniques. Certain particles of the invention are particularly suited to use where the sample to

- 4A -

be chromatographed is a macromolecule containing nucleotides, nucleosides or polypeptides, such as DNA, RNA or endotoxins.

DETAILED DESCRIPTION OF THE INVENTION

5 The invention relates to a method for making high quality adsorbent fluoropolymer particles by suspension polymerization with an aqueous solution containing a conventional dispersing agent. The basic components of the process are (1) the water-insoluble polymerization system,
10 which is comprised mainly of a polyfluorinated monomer, two or more ethylenically unsaturated monomers and a free radical-initiating catalyst, and (2) the dispersion medium, which is a dilute aqueous solution containing a conventional dispersing

15

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65
66
67
68
69
70
71
72
73
74
75
76
77
78
79
80
81
82
83
84
85
86
87
88
89
90
91
92
93
94
95
96
97
98
99
100
101
102
103
104
105
106
107
108
109
110
111
112
113
114
115
116
117
118
119
120
121
122
123
124
125
126
127
128
129
130
131
132
133
134
135
136
137
138
139
140
141
142
143
144
145
146
147
148
149
150
151
152
153
154
155
156
157
158
159
160
161
162
163
164
165
166
167
168
169
170
171
172
173
174
175
176
177
178
179
180
181
182
183
184
185
186
187
188
189
190
191
192
193
194
195
196
197
198
199
200
201
202
203
204
205
206
207
208
209
210
211
212
213
214
215
216
217
218
219
220
221
222
223
224
225
226
227
228
229
230
231
232
233
234
235
236
237
238
239
240
241
242
243
244
245
246
247
248
249
250
251
252
253
254
255
256
257
258
259
260
261
262
263
264
265
266
267
268
269
270
271
272
273
274
275
276
277
278
279
280
281
282
283
284
285
286
287
288
289
290
291
292
293
294
295
296
297
298
299
300
301
302
303
304
305
306
307
308
309
310
311
312
313
314
315
316
317
318
319
320
321
322
323
324
325
326
327
328
329
330
331
332
333
334
335
336
337
338
339
340
341
342
343
344
345
346
347
348
349
350
351
352
353
354
355
356
357
358
359
360
361
362
363
364
365
366
367
368
369
370
371
372
373
374
375
376
377
378
379
380
381
382
383
384
385
386
387
388
389
390
391
392
393
394
395
396
397
398
399
400
401
402
403
404
405
406
407
408
409
410
411
412
413
414
415
416
417
418
419
420
421
422
423
424
425
426
427
428
429
430
431
432
433
434
435
436
437
438
439
440
441
442
443
444
445
446
447
448
449
450
451
452
453
454
455
456
457
458
459
460
461
462
463
464
465
466
467
468
469
470
471
472
473
474
475
476
477
478
479
480
481
482
483
484
485
486
487
488
489
490
491
492
493
494
495
496
497
498
499
500
501
502
503
504
505
506
507
508
509
510
511
512
513
514
515
516
517
518
519
520
521
522
523
524
525
526
527
528
529
530
531
532
533
534
535
536
537
538
539
540
541
542
543
544
545
546
547
548
549
550
551
552
553
554
555
556
557
558
559
560
561
562
563
564
565
566
567
568
569
570
571
572
573
574
575
576
577
578
579
580
581
582
583
584
585
586
587
588
589
590
591
592
593
594
595
596
597
598
599
600
601
602
603
604
605
606
607
608
609
610
611
612
613
614
615
616
617
618
619
620
621
622
623
624
625
626
627
628
629
630
631
632
633
634
635
636
637
638
639
640
641
642
643
644
645
646
647
648
649
650
651
652
653
654
655
656
657
658
659
660
661
662
663
664
665
666
667
668
669
670
671
672
673
674
675
676
677
678
679
680
681
682
683
684
685
686
687
688
689
690
691
692
693
694
695
696
697
698
699
700
701
702
703
704
705
706
707
708
709
710
711
712
713
714
715
716
717
718
719
720
721
722
723
724
725
726
727
728
729
730
731
732
733
734
735
736
737
738
739
740
741
742
743
744
745
746
747
748
749
750
751
752
753
754
755
756
757
758
759
760
761
762
763
764
765
766
767
768
769
770
771
772
773
774
775
776
777
778
779
780
781
782
783
784
785
786
787
788
789
790
791
792
793
794
795
796
797
798
799
800
801
802
803
804
805
806
807
808
809
810
811
812
813
814
815
816
817
818
819
820
821
822
823
824
825
826
827
828
829
830
831
832
833
834
835
836
837
838
839
840
841
842
843
844
845
846
847
848
849
850
851
852
853
854
855
856
857
858
859
860
861
862
863
864
865
866
867
868
869
870
871
872
873
874
875
876
877
878
879
880
881
882
883
884
885
886
887
888
889
890
891
892
893
894
895
896
897
898
899
900
901
902
903
904
905
906
907
908
909
910
911
912
913
914
915
916
917
918
919
920
921
922
923
924
925
926
927
928
929
930
931
932
933
934
935
936
937
938
939
940
941
942
943
944
945
946
947
948
949
950
951
952
953
954
955
956
957
958
959
960
961
962
963
964
965
966
967
968
969
970
971
972
973
974
975
976
977
978
979
980
981
982
983
984
985
986
987
988
989
990
991
992
993
994
995
996
997
998
999
1000
1001
1002
1003
1004
1005
1006
1007
1008
1009
1010
1011
1012
1013
1014
1015
1016
1017
1018
1019
1020
1021
1022
1023
1024
1025
1026
1027
1028
1029
1030
1031
1032
1033
1034
1035
1036
1037
1038
1039
1040
1041
1042
1043
1044
1045
1046
1047
1048
1049
1050
1051
1052
1053
1054
1055
1056
1057
1058
1059
1060
1061
1062
1063
1064
1065
1066
1067
1068
1069
1070
1071
1072
1073
1074
1075
1076
1077
1078
1079
1080
1081
1082
1083
1084
1085
1086
1087
1088
1089
1090
1091
1092
1093
1094
1095
1096
1097
1098
1099
1100
1101
1102
1103
1104
1105
1106
1107
1108
1109
1110
1111
1112
1113
1114
1115
1116
1117
1118
1119
1120
1121
1122
1123
1124
1125
1126
1127
1128
1129
1130
1131
1132
1133
1134
1135
1136
1137
1138
1139
1140
1141
1142
1143
1144
1145
1146
1147
1148
1149
1150
1151
1152
1153
1154
1155
1156
1157
1158
1159
1160
1161
1162
1163
1164
1165
1166
1167
1168
1169
1170
1171
1172
1173
1174
1175
1176
1177
1178
1179
1180
1181
1182
1183
1184
1185
1186
1187
1188
1189
1190
1191
1192
1193
1194
1195
1196
1197
1198
1199
1200
1201
1202
1203
1204
1205
1206
1207
1208
1209
1210
1211
1212
1213
1214
1215
1216
1217
1218
1219
1220
1221
1222
1223
1224
1225
1226
1227
1228
1229
1230
1231
1232
1233
1234
1235
1236
1237
1238
1239
1240
1241
1242
1243
1244
1245
1246
1247
1248
1249
1250
1251
1252
1253
1254
1255
1256
1257
1258
1259
1260
1261
1262
1263
1264
1265
1266
1267
1268
1269
1270
1271
1272
1273
1274
1275
1276
1277
1278
1279
1280
1281
1282
1283
1284
1285
1286
1287
1288
1289
1290
1291
1292
1293
1294
1295
1296
1297
1298
1299
1300
1301
1302
1303
1304
1305
1306
1307
1308
1309
1310
1311
1312
1313
1314
1315
1316
1317
1318
1319
1320
1321
1322
1323
1324
1325
1326
1327
1328
1329
1330
1331
1332
1333
1334
1335
1336
1337
1338
1339
1340
1341
1342
1343
1344
1345
1346
1347
1348
1349
1350
1351
1352
1353
1354
1355
1356
1357
1358
1359
1360
1361
1362
1363
1364
1365
1366
1367
1368
1369
1370
1371
1372
1373
1374
1375
1376
1377
1378
1379
1380
1381
1382
1383
1384
1385
1386
1387
1388
1389
1390
1391
1392
1393
1394
1395
1396
1397
1398
1399
1400
1401
1402
1403
1404
1405
1406
1407
1408
1409
1410
1411
1412
1413
1414
1415
1416
1417
1418
1419
1420
1421
1422
1423
1424
1425
1426
1427
1428
1429
1430
1431
1432
1433
1434
1435
1436
1437
1438
1439
1440
1441
1442
1443
1444
1445
1446
1447
1448
1449
1450
1451
1452
1453
1454
1455
1456
1457
1458
1459
1460
1461
1462
1463
1464
1465
1466
1467
1468
1469
1470
1471
1472
1473
1474
1475
1476
1477
1478
1479
1480
1481
1482
1483
1484
1485
1486
1487
1488
1489
1490
1491
1492
1493
1494
1495
1496
1497
1498
1499
1500
1501
1502
1503
1504
1505
1506
1507
1508
1509
1510
1511
1512
1513
1514
1515
1516
1517
1518
1519
1520
1521
1522
1523
1524
1525
1526
1527
1528
1529
1530
1531
1532
1533
1534
1535
1536
1537
1538
1539
1540
1541
1542
1543
1544
1545
1546
1547
1548
1549
1550
1551
1552
1553
1554
1555
1556
1557
1558
1559
1560
1561
1562
1563
1564
1565
1566
1567
1568
1569
1570
1571
1572
1573
1574
1575
1576
1577
1578
1579
1580
1581
1582
1583
1584
1585
1586
1587
1588
1589
1590
1591
1592
1593
1594
1595
1596
1597
1598
1599
1600
1601
1602
1603
1604
1605
1606
1607
1608
1609
1610
1611
1612
1613
1614
1615
1616
1617
1618
1619
1620
1621
1622
1623
1624
1625
1626
1627
1628
1629
1630
1631
1632
1633
1634
1635
1636
1637
1638
1639
1640
1641
1642
1643
1644
1645
1646
1647
1648
1649
1650
1651
1652
1653
1654
1655
1656
1657
1658
1659
1660
1661
1662
1663
1664
1665
1666
1667
1668
1669
1670
1671
1672
1673
1674
1675
1676
1677
1678
1679
1680
1681
1682
1683
1684
1685
1686
1687
1688
1689
1690
1691
1692
1693
1694
1695
1696
1697
1698
1699
1700
1701
1702
1703
1704
1705
1706
1707
1708
1709
1710
1711
1712
1713
1714
1715
1716
1717
1718
1719
1720
1721
1722
1723
1724
1725
1726
1727
1728
1729
1730
1731
1732
1733
1734
1735
1736
1737
1738
1739
1740
1741
1742
1743
1744
1745
1746
1747
1748
1749
1750
1751
1752
1753
1754
1755
1756
1757
1758
1759
1760
1761
1762
1763
1764
1765
1766
1767
1768
1769
1770
1771
1772
1773
1774
1775
1776
1777
1778
1779
1780
1781
1782
1783
1784
1785
1786
1787
1788
1789
1790
1791
1792
1793
1794
1795
1796
1797
1798
1799
1800
1801
1802
1803
1804
1805
1806
1807
1808
1809
1810
1811
1812
1813
1814
1815
1816
1817
1818
1819
1820
1821
1822
1823
1824
1825
1826
1827
1828
1829
1830
1831
1832
1833
1834
1835
1836
1837
1838
1839
1840
1841
1842
1843
1844
1845
1846
1847
1848
1849
1850
1851
1852
1853
1854
1855
1856
1857
1858
1859
1860
1861
1862
1863
1864
1865
1866
1867
1868
1869
1870
1871
1872
1873
1874
1875
1876
1877
1878
1879
1880
1881
1882
1883
1884
1885
1886
1887
1888
1889
1890
1891
1892
1893
1894
1895
1896
1897
1898
1899
1900
1901
1902
1903
1904
1905
1906
1907
1908
1909
1910
1911
1912
1913
1914
1915
1916
1917
1918
1919
1920
1921
1922
1923
1924
1925
1926
1927
1928
1929
1930
1931
1932
1933
1934
1935
1936
1937
1938
1939
1940
1941
1942
1943
1944
1945
1946
1947
1948
1949
1950
1951
1952
1953
1954
1955
1956
1957
1958
1959
1960
1961
1962
1963
1964
1965
1966
1967
1968
1969
1970
1971
1972
1973
1974
1975
1976
1977
1978
1979
1980
1981
1982
1983
1984
1985
1986
1987
1988
1989
1990
1991
1992
1993
1994
1995
1996
1997
1998
1999
2000
2001
2002
2003
2004
2005
2006
2007
2008
2009
2010
2011
2012
2013
2014
2015
2016
2017
2018
2019
2020
2021
2022
2023
2024
2025
2026
2027
2028
2029
2030
2031
2032
2033
2034
2035
2036
2037
2038
2039
2040
2041
2042
2043
2044
2045
2046
2047
2048
2049
2050
2051
2052
2053
2054
2055
2056
2057
2058
2059
2060
2061
2062
2063
2064
2065
2066
2067
2068
2069
2070
2071
2072
2073
2074
2075
2076
2077
2078
2079
2080
2081
2082
2083
2084
2085
2086
2087
2088
2089
2090
2091
2092
2093
2094
2095
2096
2097
2098
2099
2100
2101
2102
2103
2104
2105
2106
2107
2108
2109
2110
2111
2112
2113
2114
2115
2116
2117
2118
2119
2120
2121
2122
2123
2124
2125
2126
2127
2128
2129
2130
2131
2132
2133
2134
2135
2136
2137
2138
2139
2140
2141
2142
2143
2144
2145
2146
2147
2148
2149
2150
2151
2152
2153
2154
2155
2156
2157
2158
2159
2160
2161
2162
2163
2164
2165
2166
2167
2168
2169
2170
2171
2172
2173
2174
2175
2176
2177
2178
217

agent. By water-insoluble solution, it is meant a solution sufficiently water-insoluble to permit suspension polymerization to occur. Preferred ethylenically unsaturated monomers are monomers having divinyl functionality. Non-fluorinated monomers having divinyl functionality are more preferred. Poly(vinyl alcohol) and poly(vinyl pyrrolidone) are preferred dispersing agents

A. Dispersing Agents

The polymerization of the polyfluorinated copolymer for use in the invention is conducted in the presence of a dilute aqueous solution containing a dispersing agent, for example poly(vinyl alcohol) or poly(vinyl pyrrolidone). The principal function of the dispersing agent is to adjust the interfacial surface tension between the finely dispersed water-insoluble polymerization components and the continuous aqueous medium phase. By regulating the concentration of dispersing agent dissolved in the aqueous medium, the droplet size of the dispersed polymerization system and thus the size of the resultant polymerized particles can be more finely controlled.

So long as the dispersing agent is essentially completely dissolved in the aqueous medium, a wide range of molecular weights of the dispersing agent may be used successfully in the practice of the invention. One preferred dispersing agent is PVA that is at least 80% hydrolyzed, and more preferably at least 86% hydrolyzed, with a molecular weight of at least about 1,000. The maximum usable molecular weight is a function of the ambient water solubility of the dispersing agent. For example, the molecular weight of the PVA used will ordinarily not exceed 150,000 and preferably is no higher than 100,000.

For the purposes of the invention, the concentration of PVA in the aqueous medium should be within the range of 1 to

SUBSTITUTE SHEET (RULE 26)

50 mL PVA per litre of water. Below 1 mL/L the modifying effect of the PVA is insufficient and above about 50 mL/L no further advantage is discernible. It is, of course, desirable to use lesser amounts of PVA in order to avoid energy-wasting increases in viscosity of the aqueous medium.

B. Polymerization System

1. Polyfluorinated Monomer: As set out above, the fluorine-containing comonomer must contain a plurality of fluorine (F) substituents. It is preferred that the fluorinated comonomer contains at least three F substitutions. In addition to these restrictions on its degree of fluorination, it is essential that the fluorinated comonomer be essentially completely insoluble in water under the polymerization temperatures encountered and essentially completely soluble in the other components of the dispersed polymerization system.

Suitable polyfluorinated comonomers are those containing active vinyl sites such as acrylates, methacrylates, vinyl compounds, maleates and itaconates. Among the many compounds within those categories are pentafluorostyrene, bis-hexafluoroisopropyl itaconate, bis-hexafluoroisopropyl maleate, heptadecafluorodecyl acrylate, perfluorooctyl methacrylate, 2,2,3,3-tetrafluoropropyl methacrylate, mono-trifluoroethyl itaconate, 2,2,2-trifluoroethyl maleate, vinyl benzyl perfluorooctanoate and vinyl trifluoroacetate.

2. Vinyl Comonomers: It is preferred that the comonomer component of the polyfluorinated copolymer for use in the invention be a non-fluorinated C₂₋₄ alkylene glycol ester of a C₃₋₆ acrylic acid (the cross-linking comonomer). The cross-linking comonomer must have at least two vinyl groups. Suitable comonomers having this composition are ethyleneglycol dimethacrylate, 1,3-propyleneglycol dimethacrylate, 1,4-

SUBSTITUTE SHEET (RULE 26)

butanediol dimethacrylate, ethyleneglycol itaconate, ethyleneglycol diacrylate, and ethyleneglycol dimaleate. Divinyl benzene can also be used for this purpose.

A mixture of non-fluorinated comonomers can also be used, where one non-fluorinated comonomer has at least two vinyl groups, i.e. the cross-linking comonomer, and the third monomer, i.e. co-monomer (c), is acrylic acid, methacrylic acid, or an ester of acrylic or methacrylic acid. Typical esters are the methyl, ethyl, and hydroxyethyl esters of these acids, epoxide containing esters of these acids and amine esters of these acids. Thus, a forth co-monomers selected from co-monomers (c) may be used in the synthesis.

The presence of co-monomer (c) facilitates the attachment of ligands for use in chromatographic separations by obviating the use of PVA as a linker, as described in US Patent Nos. 5,773,587 and 6,046,246, between the perfluorinated particle and the ligand. The addition of monomer (c) has little effect on the properties of the improved particles of the invention, such as stability of the particle or pore size.

We have shown that between 1 and 30% of the cross-linker ethylene glycol dimethacrylate can be replaced with a third or forth monomer selected from co-monomers (c). These co-monomers can be chosen depending on the functionality desired. For example, functional esters of acrylic and methacrylic acid can be added such as those containing hydroxyl, epoxide, amine, quarternary ammonium, sulphonic acid etc. can be used.

3. Free Radical Initiator: An essential component of the polymerization is a source of free radicals. In particular, the system must contain one or more compounds that thermally decompose under the conditions of polymerization to form free radical species. A preferred free radial agent is a

SUBSTITUTE SHEET (RULE 26)

mixture of azo-bis-isobutyronitrile (AIBN) and benzoyl peroxide (BPO). From about 10 to about 50 mg/L are needed for this purpose. It is recognized that higher concentrations are operable functionally. However, it is preferred to use as
5 small amounts as possible in order to lessen the amount of extraneous materials in the formed polymer particles.

C. Porogen

Suitable porogenic materials are those organic compounds which are (1) chemically inert with respect to the
10 other components of the polymerization phase, (2) completely soluble in the polymerization system, (3) completely insoluble in the continuous aqueous phase and (4) readily extractable from the polymerized particles at relatively low temperatures with a low molecular weight organic solvent. Dibutyl
15 phthalate, which is easily removed by washing the polymer particles with dichloromethane, is a preferred porogen for use in the invention. Other suitable porogens include toluene, isopropyl benzene, 2-methyl-4-pentanone, 2-methyl-4-pentanol and chlorobenzene.

20 D. Polymerization Procedure

The polymerization should be conducted in the essentially complete absence of air or any other source of oxygen contamination, which might lead to adverse reactions with any of the components of the polymerization system,
25 especially the monomers, crosslinking agent and free radical initiator. It has been found that the most practical way of removing and preventing the introduction of oxygen into the polymerization system is continuously to purge the polymerization reaction system before, during, and after
30 completion of the polymerization process with an inert gas. Any of the inert gases are, of course, suitable for this purpose. However, argon and nitrogen are the least expensive

SUBSTITUTE SHEET (RULE 26)

and will be preferred in most instances. Because the polymerization is conducted under very high energy mixing conditions, the method of introducing the purging gas is not particularly critical, so long as it is adequate in volume.

- 5 The dispersing agent functions principally for more precise control of interfacial tension between the dispersed monomer droplets and the aqueous continuous medium. The droplet size is controlled more dominantly by the amount of mixing energy used to disperse the polymerization system.
- 10 Thus, only comparatively low concentrations of PVA as dispersing agent are required in the aqueous medium, e.g., on the order of 1-100 g/L. A PVA concentration within the range of 0.5-40 g/L is preferred. Though higher concentrations can be used, they do not improve functionality. Because of the
- 15 necessity of forming very small droplets during the polymerization, it is, of course, desirable to avoid higher PVA concentrations which would render the aqueous medium more viscous.

- The amount of energy input into the polymerization is
- 20 primarily a function of the polymer particle size that is desired. Thus, if larger particles are sought, the degree of mixing (energy input) is lowered. If smaller particles are sought, the degree of mixing is raised. It is preferred that droplet size during polymerization be controlled to obtain
- 25 polymer particles within the range of 5-300 micrometers, 20-100 micrometers being especially preferred.

F. Particle Properties

- Ideal chromatography media need to have the following properties: (1) spherical shape; (2) high surface area;
- 30 availability of a wide range of (3) pore diameters and (4) particle diameters; (5) high pore volume; (6) high mechanical

SUBSTITUTE SHEET (RULE 26)

strength; and (7) both chemical and mechanical stability throughout the pH range to which the media are exposed in use.

Sphericity of the particles, rather than irregular, granular shapes, is advantageous for providing minimum
5 resistance to flow through a packed bed of the particles and minimum backpressure. Such regularly shaped particles are less likely to undergo densification during use.

Particle size and size distribution are also important properties of the particles of the invention. In
10 general, particles larger than about 20 micrometers facilitate lower backpressure in packed columns. Moreover, the chromatographic peak width and peak shape obtained with larger particles are usually wider than the peak width and shape obtained with particles in the range of 3-15 micrometers.
15 Narrow peak shapes are frequently desired for many types of separations.

The available surface area of polyfluorinated particles produced by the method of the invention is ordinarily preferred to be at least about 200 m²/g in order to obtain
20 higher loading of antigens on the particulate media. Nevertheless, media having much lower surface areas can readily be made according to the invention by changing the amount of porogen used in the polymerization system and decreasing the size of the particles. Concomitantly, a large pore volume of
25 at least 0.5 mL/g is needed in order to obtain a high surface area.

A wide range of pore sizes must be available for different chromatographic procedures. Large pores are needed for the efficient capture of larger molecules, such as
30 proteins, while small pores are needed for the efficient capture of small molecules. In general, the range of pore sizes may extend from below 60Å to as high as 1,000Å, 300-800Å

SUBSTITUTE SHEET (RULE 26)

being preferred. This range of sizes is quite readily available using the invention method of adjusting the relative amount and type of porogen within the formed polymer particles.

Because of the wide range of pH values at which chromatography media are used and because of the very high pH ranges that are encountered frequently to clean and regenerate them, it is necessary that they be chemically inert throughout the entire range of such pH exposures. In particular, chromatographic media must be able to withstand the high pH (12 or higher) encountered by the use of NaOH for cleaning the media particles, typically 0.1-1 normal.

G. Uses of the Particles

The adsorbent particles of the invention are quite versatile and may be used as the stationary phase for carrying out a wide variety of chromatographic separations. Examples of the chromatographic separations contemplated include reverse phase separations, affinity separations, expanded bed separations, ion-exchange chromatography, gel filtration, chromatographic component separation, solid phase extraction, filtration and other recognised technical methods of distinguishing, measuring or collecting components of a chemical, biological or physical mixture. The particles may be used as support for grafting different types of ligands. Certain of the particles are particularly suited to use where the sample to be chromatographed is DNA, RNA or polypeptides.

The polyfluorinated particles of the invention can be used for chromatographic separations either with or without a coating of a hydrophilic polymer, such as poly(vinyl alcohol).

The surface of the uncoated particles of Examples 3 and 4 is hydrophobic, but with a slight polarity, which combination of properties is ideal for reverse phase chromatographic separations. Reverse phase chromatography

SUBSTITUTE SHEET (RULE 26)

involves the use of a relatively non-polar stationary phase in conjunction with a very polar mobile phase that is usually water. This technique is used to separate solutes of lower polarity. Reverse phase chromatography is usually performed using silica that is coated with an organic silane to provide hydrophobicity. However, the hydrophobized silica has a severe limitation in that it cannot be used at pH greater than 11 and cannot be cleaned with concentrated caustic soda solutions without dissolving the particles. A substantial advantage of the polyfluorinated particles of the invention is that they do not have this limitation.

The use of the uncoated invention particles for reverse phase chromatography is illustrated by Example 28 and the stability of the particles of the invention toward basic solutions is shown by the data obtained in Example 29 below.

Suitable hydrophilic polymers for use in coating the polyfluorinated particles of the invention are those which are uncharged, water-soluble, non-cyclic and have a multiplicity of hydroxyl groups. Though many several such hydrophilic polymers are useful for this particular function, poly(vinyl alcohol) is preferred.

Advantageously, the polyfluorinated compounds of the invention may be used in medical devices with or without ligands on their surfaces to do separations that are not classified as chromatographic. For example, components of blood can be separated using a medical device in which the blood is pumped through a cartridge extra-corporeally and returned to the body. A component such as a toxin would be removed and not returned to the body.

Due to the stability of the polyfluorinated particles of the invention, sterilization can be done by gamma irradiation without destroying the particle. This property

SUBSTITUTE SHEET (RULE 26)

makes the particles particularly well suited for uses in medical devices that must be sanitized.

H. Derivatization of Particles

If desired, the PVA-coated polyfluorinated particles of Examples 5 and 6 can be functionalized by reacting suitable molecules with the hydroxyl groups of the PVA. Thus, strong cationic ion exchange functionality can be provided to the particle surfaces by placing sulfonic acid groups on the surface. Likewise, strong anionic ion exchange functionality can be provided by applying quaternary amines. Weak cation functionality can be produced by the use of carboxylic groups and weak anion functionality can be obtained by the use of primary amines.

15 EXAMPLES

Example 1: Production of a porous copolymer of ethyleneglycol dimethacrylate, pentafluorostyrene and hydroxyethylmethacrylate

Four hundred ninety mL of distilled water were placed in a vessel and agitated with a high efficiency paddle mixer at 800 rpm. With continuing agitation, argon gas was added to purge oxygen from the water and 3.9 g of poly(vinyl alcohol) were added to the water. Agitation and purging were continued for 30 minutes, during which vortexing of the mixture was reduced by changing the angle of the agitator. Ethyleneglycol dimethacrylate (50.1 g) and pentafluorostyrene (39.8 g) and hydroxyethyl methacrylate (5.6 g) were mixed together and 127 mL of dibutylphthalate were added to the mixture after which 0.48 g azo-bis-isobutyronitrile (AIBN) and 0.45 g of benzoyl peroxide (BPI) were added. The mixture was then stirred until homogeneous. The homogeneous mixture was then added rapidly to

SUBSTITUTE SHEET (RULE 26)

the aqueous poly(vinyl alcohol) solution and the resultant polymerization mixture was heated to about 80°C. Agitation at 800 rpm and argon purging were continued throughout until the polymerization was complete.

5 Upon separating the formed fluoropolymer particles from the polymerization medium, they were washed sequentially with (1) 200 mL of distilled water at 60°C, (2) 200 mL of acetone at 60°C and (3) 200 mL of a 30/70% by volume mixture of hot water and acetone at 70°C. Upon completion of the washing
10 steps, the particles were dried overnight in an oven at 70°C.

The washed and dried fluoropolymer particles were then refluxed with 10% wt. dichloromethane for 6-7 hours at 50°C to remove the porogenic material from the particles. The porogen-free particles were placed on a sintered glass funnel
15 and rinsed with 50 mL of acetone per gram of particles, after which the rinsed particles were dried overnight at 70°C.

The washed polyfluorinated particles had an average particle size of 51 micrometers, surface area of 300 m²/g and pore volume of 1.0 mL/g. This procedure was very effective in
20 making porous, spherical beads that would withstand pressure of 2000 psi in a chromatographic separation.

Variations of this Example were also performed, as follows. A porous copolymer of ethylene glycol dimethacrylate and pentafluorostyrene and epoxy ethyl methacrylate was
25 prepared by adding to 490 ml of distilled water nitrogen gas over a 30-minute period to purge the oxygen from the water. Polyvinyl alcohol (3.9 g) was added. Pentafluorostyrene (30.9 g), divinylbenzene (35.7 g), epoxy ethyl methacrylate (20.0 g) and dibutyl phthalate (127 ml) were mixed together in a
30 separate vessel. Azo-bis-isobutyronitrile (0.40 g) and benzoyl peroxide (0.30 g) were added to the mixed monomers. The

SUBSTITUTE SHEET (RULE 26)

mixture of the monomers and the peroxide catalysts was added to a stirred mixture of the water and PVA. The mixture was heated to 80 C with agitation of 800 rpm from a motor-driven, stirring paddle. The mixture was allowed to polymerize over a 4-hour
5 period after which the polymerization was considered complete. The polymer particles were separated from the water and washed and dried. The porogen was removed as described above.

The polymerization was conducted as in Example 1 only poly(vinyl pyrrolidone) was substituted for the PVA dispersing
10 agent. The polymerization proceeded as in Example 1, only the particles were more finely divided after drying. In Example 1, the particles often clumped together on drying but were easily broken apart by mechanical or ultrasonic methods. The use of poly(vinyl pyrrolidone) prevented the clumping.

15 Porous, perfluorinated, ion-exchange particles can also be made by substituting a functional co-monomer for the crosslinker, ethylene glycol dimethacrylate. An example is the substitution of 20.0 g. of methacrylic acid for the ethylene glycol dimethacrylate. The resulting polymer can function as a weak
20 cation exchanger.

Example 2: Production of a porous copolymer of ethyleneglycol dimethacrylate, 2-(N-ethyl perfluoro octane sulfo amido) perfluoromethacrylate and methacrylic acid

25 A porous copolymer of ethyleneglycol dimethacrylate, 2-(N-ethyl perfluoro octane sulfo amido) perfluoromethacrylate and methacrylic acid was prepared in the following manner:

Set-up:

1 L. cylindrical reactor fitted with a "type E"
30 agitator (Cole Palmer, 6 cm diameter and 10 cm height), reflux

SUBSTITUTE SHEET (RULE 26)

16

condenser, gas inlet tube and immersed temperature probe. The agitator is positioned so that its top impeller blade is located just above the level of the aqueous phase.

Aqueous phase:

5 3.9 g PVA (Aldrich, 85,000 to 146,000 Daltons, 97-99% hydrolyzed) in 490 mL deionized (DI) water

Organic phase:

1.7 g of polystyrene (Aldrich, 90,000 MW standard)

171 mL isopropyl benzene (Aldrich, 99%)

10 68.5 g ethylene glycol dimethacrylate (Aldrich, 98%, 100 ppm methyl ether of hydroquinone (MEHQ))

85.6 g 2-(N-ethylperfluorooctane sulphonamido) ethyl methacrylate (Monomers, Polymers and Dajack)

17.1 g of methacrylic acid

15 0.57 g AIBN (Aldrich, 99%)

1.14 g BPO (Aldrich, 98%)

The aqueous phase was prepared by predissolving the PVA in water at approximately 50°C. The aqueous phase was charged to the reactor and sparged with nitrogen for 25
20 minutes.

The polystyrene was pre-dissolved in the isopropyl benzene. The mixture of the three monomers was then added, followed by the initiators. After stirring for 1 hour, the organic phase still appeared cloudy and was added as such to
25 the reactor. Under a nitrogen sweep, the mixture was stirred at 800 rpm and heated to 80°C over a period of 30 minutes. Upon reaching reaction temperature, most of the organic phase

SUBSTITUTE SHEET (RULE 26)

agglomerated into a single mass that broke up into individual beads again after 25 minutes.

After 9 hours at reaction temperature, the system was allowed to cool, the aqueous phase siphoned out and the resin
5 beads washed with 500 mL DI water, 500 mL acetone, 500 mL acetone water (30:70), 500 mL hot water and twice with 500 mL acetone.

After air drying, the resin weight is 168 g.

The resin is refluxed for 5 hours in 1 L methylene
10 chloride, washed with 1 L acetone and air dried.

The washed and dried fluoropolymer particles were then refluxed with 10% wt. dichloromethane for 6-7 hours at 50°C to remove the porogenic material from the particles. The
15 porogen-free particles were placed on a sintered glass funnel and rinsed with 50 mL acetone per gram of particles, after which the rinsed particles were dried overnight at 70°C. The resultant porous beads had a particle size of 50 µm and a surface area of 300 m/gm.

20 Example 3: Production of a porous copolymer of ethyleneglycol dimethacrylate and pentafluorostyrene

A porous copolymer of ethyleneglycol dimethacrylate and pentafluorostyrene was prepared according to the procedure described in Example 1, mixing together 55.7 g of
25 ethyleneglycol dimethacrylate and 39.8 g of pentafluorostyrene. No ethyl methacrylate was added to the mixture. This procedure was also very effective in making spherical porous particles of pentafluorostyrene.

SUBSTITUTE SHEET (RULE 26)

Example 4: Production of a porous copolymer of ethylene glycol dimethacrylate and 2-(N-ethyl perfluoro octane sulfo amido) perfluoromethacrylate

A porous copolymer of ethylene glycol dimethacrylate
5 and 2-(N-ethyl perfluoro octane sulfo amido)
perfluoromethacrylate was prepared according to Example 2,
except that the organic phase was composed of:

- 1.7 g of polystyrene (Aldrich, 90,000 MW standard)
- 171 mL isopropyl benzene (Aldrich, 99%)
- 10 85.6 g ethylene glycol dimethacrylate (Aldrich, 98%,
100 ppm methyl ether of hydroquinone (MEHQ))
- 85.6 g 2-(N-ethylperfluorooctane sulphonamido) ethyl
methacrylate (Monomers, Polymers and Dajack)
- 0.57 g AIBN (Aldrich, 99%)
- 15 1.14 g BPO (Aldrich, 98%)

The procedure was very effective for making porous spherical
particles of perfluoromethacrylate.

Variations of Example 3 and 4 were performed to
demonstrate the flexibility of the process in making particles
20 of various pore morphologies, as illustrated in the following
table:

Porogen	% ethylene glycol in pentafluorostyrene mixture	Pore Diameter A	% ethylene glycol in methacrylate mixture	Pore Diameter A
Toluene	50	37		
Toluene	40	122		
Di-butyl phthalate	50	105	50	184

SUBSTITUTE SHEET (RULE 26)

Di-butyl phthalate	40	73	40	261
Di-butyl phthalate	30	78		
2-methyl-4-pentanone	50	339	50	46

The above table illustrates the effect that the type and amount of the porogen selected may have in different monomer systems.

5 Example 5: Coating of styrenic fluoropolymer particles with PVA

Using dry fluoropolymer particles prepared in the manner of Example 3, 50 g of such particles were de-agglomerated by sonication in methanol for 5 minutes and soaked overnight in 150 ml of methanol. This de-agglomeration step
10 was carried out in separate batches of 2 g resin in 20 mL methanol.

The methanol resin slurry was placed in a 3 L round bottom flask and enough methanol siphoned out so that it just covered the beads. A solution of 80 g PVA (31,000 to 50,000
15 Daltons, 98% hydrolyzed) in 1 L deionized water, previously prepared by dissolving the PVA at 50°C, was then added to the flask and the resulting slurry stirred at room temperature for 24 hours. After collecting a sample for PVA content analysis, the loading solution was separated from the beads by
20 decantation. The beads were transferred to a fritted funnel and washed twice for 10 minutes with 500 mL deionized water, followed by removal of the water by suction. The water washes were combined and a sample retained for PVA content analysis. The washed beads were returned to the round bottom flask, and
25 1 L of deionized water was added. Stirring was resumed, and 1 mL of 50% aqueous solution of glutaraldehyde was added.

SUBSTITUTE SHEET (RULE 26)

immediately followed by 8 mL of 5 N aqueous HCl. After stirring for an additional 24 hours at room temperature, the beads were transferred to a fritted funnel, drained, washed three times with 1 L deionized water and set aside as a wet
5 slurry.

This example shows that spherical polyfluorinated particles made in accordance with the invention can be readily coated with poly(vinyl alcohol) in this manner.

10 **Example 6: Coating of styrenic fluoropolymer particles with PVA**

Again using dry fluoropolymer particles prepared in the manner of Example 3, 50 g of the particles were soaked in methanol and coated with PVA in the manner of Example 5, except that the concentration of the PVA in the aqueous solution was
15 raised to 20 g/L.

Example 7: Coating of methacrylic fluoropolymer particles with PVA

In this Example, 50 g of fluoropolymer particle
20 prepared in the manner of Example 4 were coated with PVA in the manner of Example 5.

Example 8: Measurement of PVA coating on fluoropolymer particles

The concentration of PVA was determined by measuring
25 the absorbance of the PVA/iodine/boric acid complex measured at 690 nm and comparing it with a calibration curve prepared using standard PVA solutions. The linear range of the calorimetric assay is up to 1 mg PVA/mL. The amount of PVA adsorbed on the resin was determined by the difference of the initial coating

SUBSTITUTE SHEET (RULE 26)

solution concentration minus the final solution concentration.
Results are reported in mg or g PVA/g dry resin.

For a 9.31 mg/mL PVA coating solution, dilute samples
100X with distilled water. Pipette 2.0 mL of the samples
5 prepared in 1) into the cuvette along with 0.5 mL of the 0.6M
boric acid solution and 0.1 mL of the KI/I₂ solution. Mix and
let stand in the darkness for 30+5 min. before taking the
absorbance rating at 690 nm. Calculate the weight of PVA
adsorbed onto the fluoropolymer beads by the following
10 relationship:

$$\text{mg PVA/g resin} = \frac{(C_i)(V_i) - (C_f)(V_f)}{W}$$

where,

C_i = Concentration (mg/mL) of initial PVA coating
15 solution

V_i = Volume (mL) of PVA coating solution

C_f = Concentration (mg/mL) of PVA coating solution at
the end of coating process

V_f = Final volume (mL) of coating solution

20 V_f may be greater than V_i due to a contribution from
the wetting solvent

W = Weight (g) of dry fluoropolymer used in the
coating process

Using this method, the amount of PVA adsorbed onto
25 the perfluorinated polymers was measured at 0.4 g PVA per g of
the dry fluoropolymer prepared as in Example 5 and 1.51 g PVA
per g of the dry fluoropolymer prepared as in Example 7.

SUBSTITUTE SHEET (RULE 26)

This example shows that the polyfluorinated polymer of the invention was well coated with poly(vinyl alcohol).

Example 9: Measurement of HSA capacity of PVA-coated
5 fluoropolymer particles

Fluoropolymer prepared and coated with a high level of PVA in the manner of Example 5 was tested with respect to their human serum albumin (HSA) capacity. In particular, 4 mL of a 4 mg/mL solution of HSA in 20 mM phosphate buffer at pH
10 7.4 were added to 0.5 g of PVA coated beads prepared as in Example 5 and the resulting slurry rotated on a flat bed mixer for 16 hours at room temperature. The concentration of HSA in the supernatant was then determined using the Bradford assay. The amount of protein non-specifically bound to the resin,
15 calculated by difference, was 2 mg/g dry fluoropolymer.

This example shows clearly that protein will bind to the uncoated invention substrate more efficiently than to the corresponding coated substrate.

20 Example 10: Measurement of HSA capacity of PVA-coated fluoropolymer particles

Fluoropolymer particles prepared and coated with a low level of PVA in the manner of Example 5 were tested with respect to their HSA capacity by the same procedure as Example
25 9. The amount of HSA adsorbed was determined to be 12.5 mg/g of dry resin.

The example shows that when poly(vinyl alcohol) is coated onto the polyfluorinated particles of the invention, it is a uniform, effective coating.

SUBSTITUTE SHEET (RULE 26)

Example 11: Measurement of lysozyme capacity of PVA-coated fluoropolymer particles

Fluoropolymer particles prepared and coated with a high level of PVA in the manner of Example 6 were tested with respect to their lysozyme capacity by the same procedure as Example 9. In particular, 4 mL of a 4 mg/mL-solution of lysozyme in 20 mM carbonate buffer at pH 9.0 was added to 0.5 g of PVA coated beads prepared as in Example 6. The resulting slurry was rotated on a flat bed mixer for 16 hours at room temperature. The concentration of lysozyme in the supernatant was then determined based on the supernatant's adsorption at 280 nm. The amount of protein non-specifically bound to the fluoropolymer beads, calculated by difference, was 5 mg/g dry resin.

Example 12: Size exclusion chromatography of proteins

A 10 mL Pharmacia HR 10/30 column was packed with fluoropolymer particles prepared as in Example 5 and equilibrated with 20 mM phosphate buffer at pH 7.0. The column void volume (V_0) was determined by measuring the elution volume (V_e) of Blue Dextran 2000 (0.5 mL injection, 4 mg/mL, 20 mM phosphate buffer at pH 7.0). 0.05 mL of a 10 mg/mL each of ribonuclease A, ovalbumin and aldolase was loaded onto the column and eluted with the equilibration buffer at 0.02 mL/min. Similarly, a solution of chymotrypsinogen A and bovine serum albumin were loaded onto the column and eluted with the equilibration buffer at a flow rate of 0.02 mL/min. The elution volumes of the various proteins were measured from the chromatogram (UV detection) and their respective partition coefficients (K_{av}) calculated using the following equation:

SUBSTITUTE SHEET (RULE 26)

$$K_{av} = (V_e - V_o) / (V_t - V_o)$$

where V_t is the total volume of the column.

The results, summarized in Table 1 below, show the expected inverse relationship between partition coefficient and molecular weight for globular proteins.

Table 1

PROTEIN	MOLECULAR WEIGHT (Daltons)	PARTITION COEFFICIENT
Ribonuclease A	13,700	1
Chymotrypsinogen A	25,000	0.36
Ovalbumin	43,000	0.18
Albumin	67,000	0.13
Aldolase	158,000	0.07

Example 13: Binding of blue dye to PVA-coated fluoropolymer particles

10 This example was directed to the binding of a blue dye at low concentration on PVA-coated fluoropolymer particles.

To 1 mL of PVA-coated fluoropolymer beads prepared as in Example 5 were added a solution of 50 micromol (40 mg) of Cibacron Blue F3G-A in 8.4 mL of water and 250 microliters of 2M NaCl. After mixing for 30 minutes on a flat bed mixer, 500 micromoles of Na_2CO_3 were added and the slurry tumble-mixed for 16 hours at 80°C. The beads were then washed, retaining the filtrates, on a glass sinter with 50 mL each of water, 1 M NaCl, dimethylformamide, water 3:1 (v/v) methanol/water, water, 20 methanol, water, 1 M NaOH and finally 100 mL fractions of water until the filtrate became clear. The amount of Cibacron Blue

SUBSTITUTE SHEET (RULE 26)

F3G-A bound to the resin - 15 micromol/mL was determined by measuring the dye concentration in the washing solutions, determined by adsorbance at 620 nm, and calculating the amount bound by difference.

5

Example 14: Binding of blue dye to PVA-coated fluoropolymer particles

This example was directed to binding Cibacron Blue F3G-A dye to a PVA-coated styrenic fluoropolymer particles with a high concentration of the blue dye. The resin was coated with the PVA in the manner of Example 6, except that the amount of water to dissolve the 50 Mmoles of blue dye was 1 mL. The dye was applied in the manner of Example 13. The resulting ligand density was 25 micromoles per mL of the fluoropolymer particles.

Example 15: Lysozyme capacity of the affinity polymer

This example was carried out using a fluoropolymer prepared in the manner of Example 13, which contained a blue dye binding with low ligand density. A 1 mL Pharmacia HR 5/10 column was packed with a resin prepared as in Example 13 and equilibrated with sodium phosphate buffer (20 mM, pH 7.4). 4 mL of a 5 mg/mL solution of lysozyme in the equilibration buffer was loaded onto the resin at 1 mL/min. The lysozyme was then eluted from the resin using 1 M NaCl in 20 mM sodium phosphate buffer, pH 7.4. The amount of lysozyme eluted, as determined by measuring the eluent's absorption at 280 nm, was 18 mg per mL of fluoropolymer.

This example should be compared with Example 11 where no blue dye was bonded to the poly(vinyl alcohol).

SUBSTITUTE SHEET (RULE 26)

Example 16: Lysozyme capacity of the affinity polymer

This example was carried out using a fluoropolymer prepared in the same manner as Example 14, but having a high ligand density. The amount of lysozyme eluted was 20 mg/mL of resin.

Example 17: Non-adsorption of myoglobin by the affinity polymer

A fluoropolymer was prepared using the procedure of Example 14 using myoglobin as the protein. No protein adsorption by the polymer could be detected.

Example 18: Bed expansion

In this test, a 40 cm x 1 cm column was packed with fluoropolymer particles prepared in the manner of Example 14 and subsequently screened to a 63 to 82 micrometers particle diameter range. Water was pumped up-flow in the column and the bed expansion ration (the ratio of the bed depth at a given flow rate vs. bed depth without flow) H_e/H_o , measured at various flow rates. The results are summarized in Table 2.

TABLE 2

Flow Rate (cm/h)	8	25	40	55	68
Bed Expansion ration (H_e/H_o)	1.2	1.7	1.75	1.9	2.1

These data illustrate the advantageous use of the invention particles, which results from their higher density,

SUBSTITUTE SHEET (RULE 26)

i.e., 1.2 g/mL versus only 1.09 g/mL for prior art polymeric particles.

Examples 19 to 27: Chemical stability of the resin

5 A series of tests was carried out to determine the chemical stability of the adsorbent resin prepared in the manner of Example 3. For this series, 200 mg of fluoropolymer particles prepared as in Example 14 were soaked in 2 mL of the solvent indicated. Leakage of the Cibacron Blue F3G-A was
10 checked over time by monitoring the supernatant adsorbance at 620 nm. The dye concentrations measured in the supernatant after 37 days are summarized in Table 3.

TABLE 3

	SOLVENT (micromol)	DYE CONCENTRATION
EXAMPLE 19	25% aq. Glycerol	0.008
EXAMPLE 20	1% aq. Sodium dodec. Sulfate	0.008
EXAMPLE 21	8 M urea	0.004
EXAMPLE 22	1 M NaSCN	0.01
EXAMPLE 23	5 M HCl	0.002
EXAMPLE 24	dimethyl formamide	0.01
EXAMPLE 25	Methanol	n.d
EXAMPLE 26	Acetone	n.d
EXAMPLE 27	Water	0.002

15

SUBSTITUTE SHEET (RULE 26)

Example 28: Use of the uncoated polyfluorinated particles of the invention for reverse-phase chromatography

Polymer particles prepared in the manner of Examples 3 and 4 were packed at 1,600 psi into stainless steel columns of 250 cm length and 0.46 cm inside diameter. The slurry solvent was 50/50 by volume methanol/isopropanol. The gradient test mixture solvent was 50/50 by volume acetonitrile/water with 0.1 TFA. The mobile phase was A=water with 0.1% TFA, B=acetonitrile with 0.1% TFA. The test mixture was Vitamin B-12 (1.0 mg), bovine insulin (3.0 mg), ribonuclease A (3.0 mg), human albumin (3.0 mg) and thyroglobulin (3.0 mg). The retention times (minutes) comparing the effectiveness of methacrylic particles with the pentafluorostyrene polymer particles of the invention are set out in Table 4.

15

TABLE 4

EFFECTIVENESS OF PENTAFLUOROSTYRENE POLYMER AND
FLUORINATED METHACRYLIC POLYMER SUBSTRATES IN
REVERSE-PHASE CHROMATOGRAPHY

Solute	Pentafluorostyrene Polymer	Fluorinated Methacrylic Polymer
(Retention time, minutes)		
Vitamin B-12	1.00	1.00
Bovine insulin	1.59	1.75
Ribonuclease A	1.83	2.02
Human albumin	2.08	2.32
Thyroglobulin	2.40	2.72

20

Correlation of the data showed that smooth, symmetrical, non-overlapping curves were obtained. The data therefore clearly demonstrate that both the uncoated

SUBSTITUTE SHEET (RULE 26)

pentafluorostyrene polymer and the uncoated fluorinated methacrylic polymer particles are effective media for the chromatographic separation of mixtures of materials such as proteins.

5

Example 29:

Using the columns of Example 28 filled with the uncoated polyfluorinated resin particles, the columns were washed with 60 column volumes of 5.0 normal sodium hydroxide solution, followed by 60 column volumes of deionized water. The solutes were then reinjected and the same gradient as resulted in Example 28 was observed. In particular, the caustic-washed resin showed the same retention as the resin that had not undergone such washing, thus illustrating the robustness of the particles.

15

Example 30: Modification of synthesis variables to produce polyfluorostyrene particles of widely different particle size

TABLE 5

20 VARIATION OF PROCESS VARIABLES TO
MAKE DIVERSE PARTICLE SIZE

Particle Size, micrometers	16	120
Reactants		
Deionized water, mL	660	490
Poly(vinyl alcohol), g	16	3.9
Pentafluorostyrene, g	10	39.8
Ethyleneglycol dimethacrylate, g	14	55.7

SUBSTITUTE SHEET (RULE 26)

30

Dibutyl phthalate, mL	32	127
Azo-bis-isobutyronitrile, g	0.12	0.48
Benzyl peroxide, g	0.12	0.49
Sodium lauryl sulfate, g	0.06	None
Agitator Speed, RPM	900	395

Upon review of the performance characteristics of the adsorbents of the invention and comparison of those characteristics with the properties of other widely used adsorbent materials, it is clear from the data in Table 5 above that the polyfluorinated adsorbents of the invention are uniformly high in all the physical and chemical properties which are vital to their function.

TABLE 6

10 COMPARISON OF THE INVENTION WITH OTHER COMMERCIAL AVAILABLE CHROMATOGRAPHY SUPPORTS

Matrix	Chemical Stability (ph)	Mechanical Stability	Permeability To Macro-	Non-specific Adsorption	Ease of Derivatization	Resistance of SN NaOH
Agarose	4-9	Low	Excellent	Low	Good	Poor
Crosslinked	2-14	Low	Excellent	Low	Good	poor
Crosslinked dextran	72	Low	Poor	Low	Good	Poor
Crosslinked polyacrylamide	2-10	Medium	Poor	Low	Good	Poor
Polyacrylamide/ Dextran	3-11	Low	Excellent	Medium	Good	Poor
Polyacrylamide/ Agarose	3-10	Medium	Good	Medium	Good	Poor

SUBSTITUTE SHEET (RULE 26)

31

Crosslinked hydroxyethyl	1-14	High	Good	High	Poor	Very poor
Methacrylate	2-9	High	Good	High	Poor	Very poor
Silica						
Polystyrene/ Divinylbenzene	1-14	High	Good	High	Good	Good
Polyfluorinated particle of the invention with hydrophilic surface coating	1-14	High	Excellent	Low	Excellent	Excellent

From the data in Table 6, it can readily be seen that the adsorbents of the invention are chemically stable over a very broad pH range and have a high mechanical stability. The invention adsorbents also have excellent permeability to macromolecules and, quite desirably, low non-specific adsorption properties. In addition, the claimed adsorbents have excellent ease of derivatization and excellent resistance to the corrosive effects of 5N NaOH solutions. None of the other well-known adsorbents have such uniformly outstanding performance in all of the listed functionally important properties.

15 **Example 31: Use of the fluorinated particles of the invention in the separation of components of plasmids**

The plasmid (Amp resistant) transformed host (DH5-alpha) was grown to high density in an enriched medium and the bacterial pellet was subjected to an alkaline lysis procedure. The lysate was filtered and then precipitated with 0.7 volumes of ice cold isopropyl alcohol (IPA) by centrifugation at 8000 x

SUBSTITUTE SHEET (RULE 26)

g for 45 minutes. The liquid from the centrifugation was used as the sample to be chromatographed.

A Vantage-L series column (4.4 cm id) was packed with an ethanolic slurry containing approximately 90 ml of the particles described in Example 3 (50µm, surface area of 300/m²/gm, non-PVA coated). The column was packed at about 20 ml/min (approximately 80 cm/h linear flow rate) and operated at 16 ml/min. Column effluent was monitored at 260 nm and the absorbance was detected on a chart recorder. The column was equilibrated with EQB (0.1 M potassium phosphate, pH 7, 2 mM tetrabutylammoniumphosphate (TBAP) and 1% ethanol) and the above described sample to be chromatographed (30mg worth) was not loaded until the pH of the effluent was less than 9. The wash buffer WB1 was 93% sodium chloride/TRIS/EDTA, pH 8, and 7% ethanol. The elution buffers were the following: EL1 (elution buffer 1) was 0.1 M potassium phosphate, 2 mM TBAP, 10% ethanol; EL2 (elution buffer 2) was 0.1 M potassium phosphate, 2 mM TBAP, 12.5% ethanol; and EL3 (elution buffer 3) was 0.1 M potassium phosphate, 2 mM TBAP, 10% ethanol.

20 Particle analysis:

Sample 1 was collected during the load and re-equilibration step. No DNA was present on particles in the packed column.

Sample 2 was collected while WB1 was passing through the column and contained the bulk of the RNA and a small amount of nicked open circular DNA.

Sample 3 and 4 were collected during WB1. Sample 3 contained a small amount of supercoiled DNA, more nicked/open circular DNA and the last of the RNA. Sample 4 contained a small quantity of DNA.

SUBSTITUTE SHEET (RULE 26)

- 33 -

DNA loss may be reduced by cutting back on the ethanolic content of WB1 or increasing the TBAP concentration, the latter of which is preferred since this may still allow species selectivity by ethanol concentration at samples 4, 5 and 7.

Sample 5 (EL1) contained supercoiled DNA and trace amounts of non-supercoiled.

Sample 6 (EL2) contained the bulk of the DNA of which more than 90% was supercoiled.

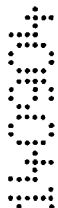
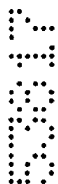
- 10 Sample 7 (EL3) contained the residual DNA of which at least 25% was non-supercoiled.

Example 32: A porous copolymer of divinyl benzene, pentafluorostyrene was prepared in the following manner

- To 490 ml of distilled water was added nitrogen gas over a 15 30-minute period to purge the oxygen from the water. Poly vinyl alcohol (3.9 g) was added. Pentafluorostyrene (30.9 g), divinylbenzene (55.0 g) and dibutyl phthalate (127 ml) were mixed together. Azo-bis-isobutyronitrile (0.40 g) and benzoyl peroxide (0.30 g) was added to the mixed monomers.
- 20 The mixture of the monomers and the peroxide catalysts was added to a stirred mixture of the water and PVA. The mixture was heated to 80°C with agitation of 800 rpm from a motor-driven, stirring paddle. The mixture was allowed to polymerize over a 4-hour period after which the
- 25 polymerization was considered complete. The polymer particles were separated from the water and washed and dried. The porogen was removed as Example 1. The resulting particles were porous and had particle size of 50 µm.

- 34 -

Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", and variations such as "comprises" and "comprising", will be understood to imply the inclusion of a
5 stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.



- 35 -

The claims defining the invention are as follows:

1. A process for the preparation of porous spherical particles of fluorinated polymer adsorbent comprising the steps:

- 5 (1) forming a water-insoluble solution of organic compounds comprising (a) a monomer selected from C_{2-4} alkylene glycol esters of a C_{3-6} acrylic acid and a divinyl benzene; (b) a polyfluorinated vinyl monomer; (c) a monomer selected from acrylic acid, methacrylic acid and esters thereof; (d) a free radical initiator; and (e) a water-insoluble, organic solvent-soluble porogenic material, the weight ratio of comonomers (a) plus (b) plus (c) to the porogenic material being from 0.5:1 to 2:1;
- 15 (2) forming a dilute solution of a dispersing agent in water from which any oxygen has been purged with inert gas;
- (3) with agitation and inert gas purging rapidly dispersing the water-insoluble solution of organic compounds from step (1) into the dilute aqueous solution from step (2) and, as necessary, adjusting the temperature of the dispersion to 30-90°C to initiate copolymerization of the monomers, the level of mixing energy being sufficient to disperse the water-insoluble solution of organic compounds in the solution from step (2) in the form of liquid droplets having an average diameter of no more than 10-300 micrometers, at least 90% of the droplets being within 40% above or below the average mean particle diameter;
- 25 (4) continuing the agitation and oxygen purging of the dispersion from step (3) for a time sufficient to effect complete copolymerization of the monomers and particulation

- 36 -

of the droplets in the form of finely divided polymer particles by precipitation of the copolymer therein;

(5) separating the finely divided copolymer particles from the polymerization reaction medium;

5 (6) extracting the porogenic material from the separated copolymer particles of step (5) by washing the particles with inert organic solvent, thereby forming pores within the copolymer; and

(7) drying the porous copolymer particles.

10 2. A process according to claim 1 wherein the monomer (a) is selected from ethyleneglycol dimethacrylate, 1,3-propyleneglycol dimethacrylate, 1,4-butanediol dimethacrylate, ethyleneglycol itaconate, ethyleneglycol diacrylate, and ethyleneglycol dimaleate.

15 3. A process according to either claim 1 or claim 2 wherein the monomer (b) is perfluorinated.

4. A process according to any one of the preceding claims wherein the monomer (b) is selected from pentafluorostyrene, bis-hexafluoroisopropyl itaconate, bis-hexafluoroisopropyl
20 maleate, heptadecafluorodecyl acrylate, perfluorooctyl methacrylate, 2,2,3,3-tetrafluoropropyl methacrylate, mono-trifluoroethyl itaconate, 2,2,2-trifluoroethyl maleate, vinyl benzyl perfluorooctanoate and vinyl trifluoroacetate.

5. A process according to any one of the preceding claims
25 wherein the monomer (c) is selected from acrylic acid, methacrylic acid, methyl-ethyl-, and hydroxyethyl- esters of acrylic acid or methacrylic acid, epoxide containing esters of acrylic acid or methacrylic acid, and amine esters of

- 37 -

acrylic acid or methacrylic acid.

6. A process according to any one of the preceding claims wherein the porogenic material (e) is selected from dibutyl phthalate, isopropyl benzene, toluene, 2-methyl-4-pentanone,
5 2-methyl-4-pentanol, chlorobenzene and mixtures thereof.

7. A process according to any one of the preceding claims, which is carried out at a temperature of 70-90°C.

8. A water-insoluble solution of organic compounds comprising (a) a monomer selected from C₂₋₄ alkylene glycol
10 esters of a C₃₋₆ acrylic acid and a divinyl benzene; (b) a polyfluorinated vinyl monomer; (c) a monomer selected from acrylic acid, methacrylic acid and esters thereof; (d) a free radical initiator; and (e) a water-insoluble, organic solvent-soluble porogenic material, the weight ratio of
15 comonomers (a) plus (b) plus (c) to the porogenic material being from 0.5:1 to 2:1.

9. A solution according to claim 8, wherein the monomer (a) is as defined in claim 2.

10. A solution according to either claim 8 or claim 9,
20 wherein the monomer (b) is as defined in claim 3 or claim 4.

11. A solution according to any one of claims 8 to 10, wherein the monomer (c) is as defined in claim 5.

12. A solution according to any one of claims 8 to 11, wherein the material (e) is as defined in claim 6.

25 13. Particles obtainable polymerization of a solution according to any one of claims 8 to 12.

-38-

14. Particles obtainable by a process according to any one of claims 1 to 7.

15. Particles according to either claim 13 or claim 14, coated with a hydrophilic polymer.

5 16. Particles according to claim 15, wherein the hydrophilic polymer is poly(vinyl alcohol).

17. A method for the chromatographic separation of separable components of a liquid solution, comprising passing the liquid solution through a bed of particles
10 according to any one of claims 13 to 16.

18. The method of claim 17, which is a reverse phase separation.

19. The method of claim 17, which is an affinity separation.

15 20. The method of claim 17, which is conducted in an expanded bed.

21. The method of any one of claims 17 to 20 which comprises adsorption on the particles of a macromolecule selected from nucleotides, nucleosides and polypeptides.

20 22. A medical device comprising particles according to either claim 15 or claim 16.

23. A process according to claim 1, substantially as hereinbefore described with reference to the examples.

25

- 39 -

24. A water-insoluble solution of organic compounds according to claim 8, substantially as hereinbefore described with reference to the examples.

5 DATED this 14th day of September, 2004.

Prometic Biosciences Inc.

by DAVIES COLLISON CAVE

Patent Attorneys for the Applicant

1
2
3
4
5
6
7
8
9
10