



US00RE43122E

(19) **United States**
(12) **Reissued Patent**
Harrison et al.

(10) **Patent Number:** **US RE43,122 E**
(45) **Date of Reissued Patent:** **Jan. 24, 2012**

(54) **APPARATUS AND METHOD FOR TRAPPING BEAD BASED REAGENTS WITHIN MICROFLUIDIC ANALYSIS SYSTEMS**

(75) Inventors: **D. Jed Harrison**, Alberta (CA); **Richard Oleschuk**, Ontario (CA); **Loranelle L. Lockyear**, McPherson, KS (US); **Cameron Skinner**, Quebec (CA); **Paul Li**, British Columbia (CA)

(73) Assignee: **The Governors of the University of Alberta**, Edmonton, Alberta (CA)

4,113,665 A	9/1978	Law et al.
4,847,120 A	7/1989	Gent
4,963,498 A	10/1990	Hillman et al.
5,275,645 A	1/1994	Ternoir et al.
5,376,252 A	12/1994	Ekström et al.
5,387,505 A	2/1995	Wu
5,453,163 A	9/1995	Yan
5,482,836 A	1/1996	Cantor et al.
5,571,410 A	11/1996	Swedberg et al.
5,587,128 A	12/1996	Wilding et al.
5,635,358 A	6/1997	Wilding et al.
5,639,428 A	6/1997	Cottingham
5,705,813 A	1/1998	Apffel et al.
5,726,026 A	3/1998	Wilding et al.

(Continued)

(21) Appl. No.: **12/820,390**

(22) Filed: **Jun. 22, 2010**

Related U.S. Patent Documents

Reissue of:

(64) Patent No.: **6,432,290**
 Issued: **Aug. 13, 2002**
 Appl. No.: **09/450,272**
 Filed: **Nov. 26, 1999**

(51) **Int. Cl.**
G01N 27/26 (2006.01)
G01N 33/00 (2006.01)

(52) **U.S. Cl.** **204/453**; 204/455; 204/451; 204/601; 204/604; 204/605; 422/502; 422/503; 422/513

(58) **Field of Classification Search** 204/450, 204/451, 455, 453, 600, 601, 604, 605; 210/656; 422/502, 503, 513

See application file for complete search history.

(56) **References Cited**

U.S. PATENT DOCUMENTS

3,352,643 A	11/1967	Ando et al.
3,433,257 A	3/1969	Jensen
3,568,692 A	3/1971	Metzger et al.

FOREIGN PATENT DOCUMENTS

EP 0459241 B1 12/1991

(Continued)

OTHER PUBLICATIONS

JPO computer generated English language translation of JP 08-327594 A, patent published on Dec. 13, 1996.*

(Continued)

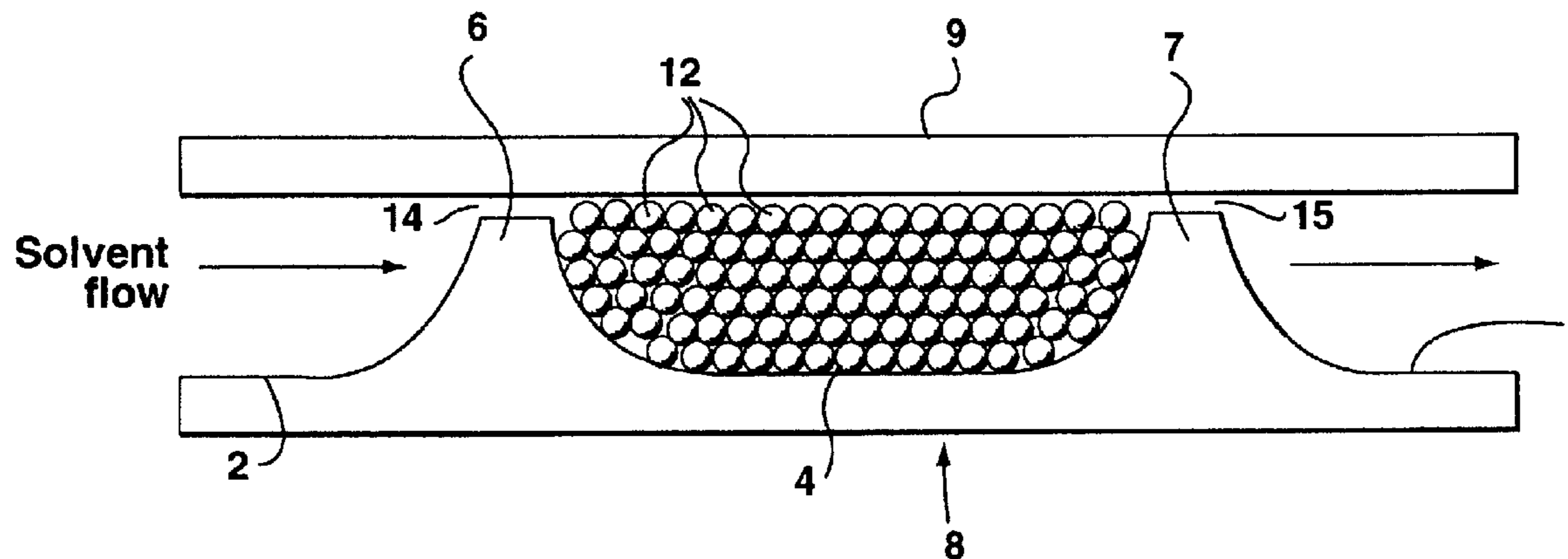
Primary Examiner — Alex Noguerola

(74) *Attorney, Agent, or Firm* — Wilson Sonsini Goodrich & Rosati

(57) **ABSTRACT**

The present invention provides an on-chip packed reactor bed design that allows for an effective exchange of packing materials such as beads at a miniaturized level. The present invention extends the function of microfluidic analysis systems to new applications including on-chip solid phase extraction (SPE) and on-chip capillary electrochromatography (CEC). The design can be further extended to include integrated packed bed immuno- or enzyme reactors.

30 Claims, 14 Drawing Sheets



US RE43,122 E

Page 2

U.S. PATENT DOCUMENTS							
5,741,462	A	4/1998	Nova et al.	6,829,753	B2	12/2004	Lee et al.
5,750,015	A	5/1998	Soane et al.	6,833,246	B2	12/2004	Balasubramanian
5,770,029	A	6/1998	Nelson et al.	6,852,287	B2	2/2005	Ganesan
5,775,371	A	7/1998	Pan et al.	6,870,185	B2	3/2005	Roach et al.
5,776,748	A	7/1998	Singhvi et al.	6,885,982	B2	4/2005	Harris et al.
5,830,662	A	11/1998	Soares et al.	6,899,137	B2	5/2005	Unger et al.
5,842,787	A	12/1998	Kopf-sill et al.	6,923,907	B2	8/2005	Hobbs et al.
5,856,174	A	1/1999	Lipshutz et al.	6,929,030	B2	8/2005	Unger et al.
5,863,502	A	1/1999	Southgate et al.	6,951,632	B2	10/2005	Unger et al.
5,872,010	A	2/1999	Karger et al.	6,953,058	B2	10/2005	Fernandes et al.
5,885,470	A	3/1999	Parce et al.	6,960,437	B2	11/2005	Enzelberger et al.
5,908,552	A	6/1999	Zimmermann et al.	6,994,986	B2	2/2006	Swartz et al.
5,922,591	A	7/1999	Anderson et al.	7,005,292	B2	2/2006	Wilding et al.
5,942,443	A	8/1999	Parce et al.	7,005,493	B2	2/2006	Huang et al.
5,948,684	A	9/1999	Weigl et al.	7,015,030	B1	3/2006	Fouillet et al.
5,971,158	A	10/1999	Yager et al.	7,046,357	B2	5/2006	Weinberger et al.
6,001,229	A	12/1999	Ramsey	7,118,910	B2	10/2006	Unger et al.
6,007,690	A	12/1999	Nelson et al.	7,198,759	B2	4/2007	Bryning et al.
6,007,775	A	12/1999	Yager	7,232,656	B2	6/2007	Balasubramanian et al.
6,010,607	A	1/2000	Ramsey	7,244,961	B2	7/2007	Jovanovich et al.
6,048,100	A	4/2000	Thrall et al.	7,258,774	B2	8/2007	Chou et al.
6,056,860	A	5/2000	Amigo et al.	7,279,146	B2	10/2007	Nassef et al.
6,073,482	A	6/2000	Moles	7,282,361	B2	10/2007	Hodge
6,074,827	A	6/2000	Nelson et al.	7,312,085	B2	12/2007	Chou et al.
6,103,199	A	8/2000	Bjornson et al.	7,312,611	B1	12/2007	Harrison et al.
6,120,184	A	9/2000	Laurence et al.	7,323,305	B2	1/2008	Leamon et al.
6,136,212	A	10/2000	Mastrangelo et al.	7,438,856	B2	10/2008	Jedrzejewski et al.
6,168,948	B1	1/2001	Anderson et al.	7,445,926	B2	11/2008	Mathies et al.
6,176,962	B1	1/2001	Soane et al.	7,488,603	B2	2/2009	Gjerde et al.
6,190,616	B1	2/2001	Jovanovich et al.	7,526,741	B2	4/2009	Lee et al.
6,197,595	B1	3/2001	Anderson et al.	7,575,865	B2	8/2009	Leamon et al.
6,207,031	B1	3/2001	Adourian et al.	7,745,207	B2	6/2010	Jovanovich et al.
6,235,471	B1	5/2001	Knapp et al.	7,763,453	B2	7/2010	Clemmens et al.
6,238,538	B1	5/2001	Parce et al.	7,766,033	B2	8/2010	Mathies et al.
6,251,343	B1	6/2001	Dubrow et al.	7,799,553	B2	9/2010	Mathies et al.
6,280,589	B1	8/2001	Manz et al.	7,943,305	B2	5/2011	Korlach et al.
6,319,476	B1	11/2001	Victor, Jr. et al.	7,959,875	B2	6/2011	Zhou et al.
6,321,791	B1	11/2001	Chow	7,976,789	B2	7/2011	Kenis et al.
6,322,683	B1	11/2001	Wolk et al.	2001/0041357	A1	11/2001	Fouillet et al.
6,348,318	B1	2/2002	Valkirs	2002/0022587	A1	2/2002	Ferguson et al.
6,379,929	B1	4/2002	Burns et al.	2002/0025529	A1	2/2002	Quake et al.
6,387,707	B1	5/2002	Seul et al.	2002/0025576	A1	2/2002	Northrup et al.
6,403,338	B1	6/2002	Knapp et al.	2002/0047003	A1	4/2002	Bedingham et al.
6,408,878	B2	6/2002	Unger et al.	2002/0048536	A1	4/2002	Bergh et al.
6,423,536	B1	7/2002	Jovanovich et al.	2002/0051992	A1	5/2002	Bridgham et al.
6,429,025	B1	8/2002	Parce et al.	2002/0058332	A1	5/2002	Quake et al.
6,432,191	B2	8/2002	Schutt	2002/0068357	A1	6/2002	Mathies et al.
6,432,290	B1	8/2002	Harrison et al.	2002/0098097	A1	7/2002	Singh
6,454,924	B2	9/2002	Jedrzejewski et al.	2002/0110900	A1	8/2002	Jovanovich et al.
6,489,112	B1	12/2002	Hadd et al.	2002/0119480	A1	8/2002	Weir et al.
6,521,188	B1	2/2003	Webster	2002/0119482	A1	8/2002	Nelson et al.
6,524,456	B1	2/2003	Ramsey et al.	2002/0139084	A1	10/2002	Tobolka
6,532,997	B1	3/2003	Bedingham et al.	2002/0160361	A1	10/2002	Loehrlein et al.
6,533,914	B1	3/2003	Liu	2003/0008308	A1	1/2003	Enzelberger et al.
6,537,757	B1	3/2003	Langmore et al.	2003/0021734	A1	1/2003	Vann et al.
6,544,734	B1	4/2003	Briscoe et al.	2003/0029724	A1	2/2003	Derand et al.
6,551,839	B2	4/2003	Jovanovich et al.	2003/0070677	A1	4/2003	Handique et al.
6,581,441	B1	6/2003	Paul	2003/0095897	A1	5/2003	Grate et al.
6,605,454	B2	8/2003	Barenburg et al.	2003/0217923	A1	11/2003	Harrison et al.
6,613,525	B2	9/2003	Nelson et al.	2004/0014091	A1	1/2004	Duck et al.
6,614,228	B2	9/2003	Hofmann et al.	2004/0018611	A1	1/2004	Ward et al.
6,618,679	B2	9/2003	Loehrlein	2004/0021068	A1	2/2004	Staats
6,623,613	B1	9/2003	Mathies et al.	2004/0037739	A1	2/2004	Mcneely et al.
6,627,446	B1	9/2003	Roach et al.	2004/0038385	A1	2/2004	Langlois et al.
6,629,820	B2	10/2003	Kornelsen	2004/0053290	A1	3/2004	Terbrueggen et al.
6,632,619	B1	10/2003	Harrison et al.	2004/0063217	A1	4/2004	Webster et al.
6,632,655	B1	10/2003	Mehta et al.	2004/0072278	A1	4/2004	Chou et al.
6,663,833	B1	12/2003	Stave et al.	2004/0008687	A1	5/2004	Childers et al.
6,685,442	B2	2/2004	Chinn et al.	2004/0086872	A1	5/2004	Childers et al.
6,752,922	B2	6/2004	Huang et al.	2004/0132170	A1	7/2004	Storek et al.
6,764,648	B1	7/2004	Roach et al.	2004/0151629	A1	8/2004	Pease et al.
6,782,746	B1	8/2004	Hasselbrink et al.	2004/0197845	A1	10/2004	Hassibi et al.
6,786,708	B2	9/2004	Brown et al.	2004/0200724	A1	10/2004	Fujii et al.
6,787,111	B2	9/2004	Roach et al.	2004/0209354	A1	10/2004	Mathies et al.
6,793,753	B2	9/2004	Unger et al.	2004/0224380	A1	11/2004	Chou et al.
6,802,342	B2	10/2004	Fernandes et al.	2005/0026300	A1	2/2005	Samper et al.
6,803,019	B1	10/2004	Bjornson et al.	2005/0047967	A1	3/2005	Chuang et al.
6,824,663	B1	11/2004	Boone	2005/0053952	A1	3/2005	Hong et al.
				2005/0142663	A1	6/2005	Parthasarathy et al.

US RE43,122 E

Page 3

2005/0161326	A1	7/2005	Morita et al.	EP	1658890	B1	5/2008
2005/0161669	A1	7/2005	Jovanovich et al.	JP	08-327594	A *	12/1996
2005/0224134	A1	10/2005	Yin et al.	JP	408327594	A *	12/1996
2005/0224352	A1	10/2005	Harrison et al.	JP	2005-337415		12/2005
2005/0241941	A1	11/2005	Parce et al.	WO	WO 96/04547	A1	2/1996
2005/0255000	A1	11/2005	Yamamoto et al.	WO	WO 98/52691		5/1998
2005/0255003	A1	11/2005	Summersgill et al.	WO	WO 98/52691	A1	11/1998
2005/0255007	A1	11/2005	Yamada et al.	WO	WO 98/53300	A2	11/1998
2005/0287572	A1	12/2005	Mathies et al.	WO	WO 98/53300	A3	2/1999
2006/0027456	A1	2/2006	Harrison et al.	WO	WO 99/36766	A1	7/1999
2006/0057209	A1	3/2006	Chapman et al.	WO	WO 99/40174	A1	8/1999
2006/0073484	A1	4/2006	Mathies et al.	WO	WO 00/40712	A1	7/2000
2006/0076068	A1	4/2006	Young et al.	WO	WO 00/60362	A1	10/2000
2006/0140051	A1	6/2006	Kim et al.	WO	WO 00/61198	A1	10/2000
2006/0163143	A1	7/2006	Chirica et al.	WO	WO 01/38865	A1	5/2001
2006/0186043	A1	8/2006	Covey et al.	WO	WO 01/85341	A1	11/2001
2006/0266645	A1	11/2006	Chen et al.	WO	WO 02/43615	A2	6/2002
2007/0015179	A1	1/2007	Klapperich et al.	WO	WO 02/043615	A3	3/2003
2007/0017812	A1	1/2007	Bousse	WO	WO 03/085379	A2	10/2003
2007/0031865	A1	2/2007	Willoughby	WO	WO 03/085379	A3	12/2003
2007/0034025	A1	2/2007	Pant et al.	WO	WO 2004/098757	A2	11/2004
2007/0105163	A1	5/2007	Grate et al.	WO	WO 2005/075081	A1	8/2005
2007/0122819	A1	5/2007	Wu et al.	WO	WO 2005/121308	A1	12/2005
2007/0175756	A1	8/2007	Nguyen et al.	WO	WO 2004/098757	A3	5/2006
2007/0184463	A1	8/2007	Molho et al.	WO	WO 2007/082480	A1	7/2007
2007/0202531	A1	8/2007	Grover	WO	WO 2008/039875	A1	4/2008
2007/0237686	A1	10/2007	Mathies et al.	WO	WO 2008/115626	A2	9/2008
2007/0238109	A1	10/2007	Min et al.	WO	WO 2008/115626	A3	11/2008
2007/0248958	A1	10/2007	Jovanovich et al.	WO	WO 2009/015296	A1	1/2009
2007/0297947	A1	12/2007	Sommers et al.	WO	WO 2009/108260	A2	9/2009
2008/0014576	A1	1/2008	Jovanovich et al.	WO	WO 2009/129415	A1	10/2009
2008/0014589	A1	1/2008	Link et al.	WO	WO 2009/108260	A3	12/2009
2008/0047836	A1	2/2008	Strand et al.	WO	WO 2010/041174	A1	4/2010
2008/0064610	A1	3/2008	Lipovsek et al.	WO	WO 2010/042784	A2	4/2010
2008/0124723	A1	5/2008	Dale et al.	WO	WO 2010/042784	A3	7/2010
2008/0138809	A1	6/2008	Kapur et al.	WO	WO 2010/141921	A1	12/2010
2008/0164155	A1	7/2008	Pease et al.				
2008/0179255	A1	7/2008	Jung et al.				
2008/0237146	A1	10/2008	Harrison et al.				
2008/0242560	A1	10/2008	Gunderson et al.				
2008/0281090	A1	11/2008	Lee et al.				
2008/0302732	A1	12/2008	Soh et al.				
2008/0311585	A1	12/2008	Gao et al.				
2009/0004494	A1	1/2009	Blenke et al.				
2009/0011959	A1	1/2009	Costa et al.				
2009/0035770	A1	2/2009	Mathies et al.				
2009/0053799	A1	2/2009	Chang-yen et al.				
2009/0056822	A1	3/2009	Young et al.				
2009/0060797	A1	3/2009	Mathies et al.				
2009/0084679	A1	4/2009	Harrison et al.				
2009/0092970	A1	4/2009	Williams				
2009/0134069	A1	5/2009	Handique				
2009/0137413	A1	5/2009	Mehta et al.				
2009/0148933	A1	6/2009	Battrell et al.				
2009/0181411	A1	7/2009	Battrell et al.				
2009/0253181	A1	10/2009	Vangbo et al.				
2009/0269504	A1	10/2009	Liao				
2009/0286327	A1	11/2009	Cho et al.				
2009/0311804	A1	12/2009	Mcbrady et al.				
2009/0314972	A1	12/2009	Mcavoy et al.				
2010/0068723	A1	3/2010	Jovanovich et al.				
2010/0129810	A1	5/2010	Greiner et al.				
2010/0165784	A1	7/2010	Jovanovich et al.				
2010/0197507	A1	8/2010	Rothberg et al.				
2010/0285578	A1	11/2010	Selden et al.				
2010/0285975	A1	11/2010	Mathies et al.				
2010/0303687	A1	12/2010	Blaga et al.				
2010/0326826	A1	12/2010	Harrison et al.				
2011/0005932	A1	1/2011	Jovanovich et al.				
2011/0027913	A1	2/2011	Bau et al.				
2011/0126910	A1	6/2011	May				
2011/0172403	A1	7/2011	Harrold				

FOREIGN PATENT DOCUMENTS

EP	0637999	A1	2/1995
EP	0527905	B1	11/1995
EP	1065378	B1	4/2002
EP	1411340	A2	4/2004
EP	1411340	A3	5/2004

OTHER PUBLICATIONS

U.S. Appl. No. 12/026,510, filed Feb. 5, 2008, Jovanovich et al.
 U.S. Appl. No. 12/526,015, filed Nov. 3, 2010, Jovanovich et al.
 U.S. Appl. No. 12/815,685, filed Jun. 15, 2010, Jovanovich et al.
 U.S. Appl. No. 12/845,650, filed Jul. 28, 2010, Jovanovich et al.
 U.S. Appl. No. 12/852,370, filed Aug. 6, 2010, Harrison et al.
 U.S. Appl. No. 12/949,623, filed Nov. 18, 2010, Kobrin et al.
 Amendment and Request for Correction of Inventorship mailed Jan. 10, 2008 in U.S. Appl. No. 10/750,533.
 Anderson, et al. A miniature integrated device for automated multistep genetic assays. *Nucleic Acids Research*. 2000;28:e60.
 Bings, et al. Microfluidic Devices Connected to Fused-Silica Capillaries with Minimal Dead Volume. *Analytical Chemistry*. 1999;71(15):3292-3296.
 Blazej, et al. Microfabricated bioprocessor for integrated nanoliter-scale Sanger DNA sequencing. *Proc. Natl. Acad. Sci. USA* 2006;103:7240-7245.
 Blazej, et al. Polymorphism Ratio Sequencing: A New Approach for Single Nucleotide Polymorphism Discovery and Genotyping. *Genome Research*. 2003;13:287-293.
 Brenner, et al. Gene expression analysis by massively parallel signature sequencing (MPSS) on microbead arrays. *Nature Biotechnology*. 2000;18(6):630-634.
 Buchholz, et al. The use of light scattering for precise characterization of polymers for DNA sequencing by capillary electrophoresis. *Electrophoresis*. 2001;22:4118-4128.
 Caplus abstract of Krohkin et al. Modified silica as a stationary phase for ion chromatography. *Journal of Chromatography A*. 1995;706:93-8.
 Chan, et al. Microfabricated Polymer Devices for Automated Sample Delivery of Peptides for Analysis by Electrospray Ionization Tandem Mass Spectrometry. *Analytical Chemistry*. 1999;71(20):4437-4444.
 Chiem, et al. Microchip systems for immunoassay: an integrated immunoreactor with electrophoretic separation for serum theophylline determination. *Clinical Chemistry*. 1998;44(3):591-598.
 Chiem, et al. Room temperature bonding of micromachined glass devices for capillary electrophoresis. *Sensors and Actuators*. 2000;B63(3):147-152.

- Coleman, et al. A sequential injection microfluidic mixing strategy. *Microfluidics and Nanofluidics*. 2005;319-327.
- Curcio, et al. Continuous Segmented-Flow Polymerase Chain Reaction for High-Throughput Miniaturized DNA Amplification. *Analytical Chemistry*. 2003;75(1):1-7.
- Datasheet Cycle Sequencing, Retrieved from the internet, URL: <http://answers.com/topic/cycle-sequencing>. Printed Sep. 3, 2010, pp. 1-2.
- Diehl, et al. BEAMing: single-molecule PCR on microparticles in water-in-oil emulsions. *Nature Methods*. 2006;3(7):551-9.
- Doherty, et al. Sparsely Cross-linked "Nanogel" Matrices as Fluid, Mechanically Stabilized Polymer Networks for High-Throughput Microchannel DNA Sequencing. *Analytical Chemistry*. 2004;76:5249-5256.
- Doherty, et al. Sparsely cross-linked "nanogels" for microchannel DNA sequencing. *Electrophoresis*. 2003;24(24):4170-4180.
- Dorfman, et al. Contamination-Free Continuous Flow Microfluidic Polymerase Chain Reaction for Quantitative and Clinical Applications. *Analytical Chemistry*. 2005;77(11):3700-3704.
- Doyle, et al. Self-Assembled Magnetic Matrices for DNA Separation Chips. *Science*. 2000;295:2237.
- Dressman, et al. Transforming single DNA molecules into fluorescent magnetic particles for detection and enumeration of genetic variations. *Proc Natl Acad Sci USA*. 2003;100(15):8817-8822.
- Emrich, et al. Microfabricated 384-Lane Capillary Array Electrophoresis Bioanalyzer for Ultrahigh-Throughput Genetic Analysis. *Analytical Chemistry*. 2002;74(19):5076-5083.
- Ericson, et al. Electroosmosis- and Pressure-Driven Chromatography in Chips Using Continuous Beds. *Analytical Chemistry*. 2000;72(1):81-87.
- European search report dated Dec. 18, 2009 for Application No. 03808583.3.
- European search report dated Sep. 1, 2010 for Application No. 5804847.1.
- Ewing, et al. Base-Calling of Automated Sequencer Traces Using Phred. I. Accuracy Assessment. *Genome Research*. 1998;8:175-185.
- Ewing, et al. Base-Calling of Automated Sequencer Traces Using Phred. II. Error probabilities. *Genome Research*. 1998;8:186-194.
- Figeys, et al. A Microfabricated Device for Rapid Protein Identification by Microelectrospray Ion Trap Mass Spectrometry. *Analytical Chemistry*. 1997;69(16):3153-3160.
- Figeys, et al. An Integrated Microfluidics-Tandem Mass Spectrometry System for Automated Protein Analysis. *Analytical Chemistry*. 1998;70(18):3728-3734.
- Figeys, et al. Microfabricated Device Coupled with an Electrospray Ionization Quadrupole Time-of-Flight Mass Spectrometer: Protein Identifications Based on Enhanced-Resolution Mass Spectrometry and Tandem Mass Spectrometry Data. *Rapid Communications in Mass Spectrometry*. 1998;12:1435-1444.
- Figeys, et al. Nanoflow Solvent Gradient Delivery from a Microfabricated Device for Protein Identifications by Electrospray Ionization Mass Spectrometry. *Analytical Chemistry*. 1998;70(18):3721-3727.
- Francis, et al. Flow analysis based on a pulsed flow of solution: theory, instrumentation and applications. *Talanta*. 2002;58(6):1029-1042.
- Ghadessy, et al. Directed evolution of polymerase function by compartmentalized self-replication. *Proc Natl Acad Sci USA*. 2001;98:4552-4557.
- Giddings, et al. A software system for data analysis in automated DNA sequencing. *Genome Research*. 1998;8:644-665.
- Goll, et al. Microvalves with bistable buckled polymer diaphragms. *Journal of Micromechanics and Microengineering*. 1996;6:77-79.
- Grover, et al. An integrated microfluidic processor for single nucleotide polymorphism-based DNA computing. *Lab on a Chip*. 2005;5(10):1033-1040.
- Grover, et al. Development and multiplexed control of latching pneumatic valves using microfluidic logical structures. *Lab on a chip*. 2006;6:623-631.
- Grover, et al. Monolithic membrane valves and diaphragm pumps for practical large-scale integration into glass microfluidic devices. *Sensors and Actuators*. 2003;B89:315-323.
- Grover, et al. Practical Valves and Pumps for Large-Scale Integration into Microfluidic Analysis Devices. *Micro Total Analysis Systems*. 2002;1:136-138.
- Hansen, et al. A robust and scalable microfluidic metering method that allows protein crystal growth by free interface diffusion. *Proc Natl Acad Sci USA*. 2002;99(26):16531-16536.
- Harrison, et al. Micromachining a Miniaturized Capillary Electrophoresis-Based Chemical Analysis System on a Chip. *Science*. 1993;261(5123):895-897.
- Hayes, et al. EDGE: A Centralized Resource for the Comparison, Analysis, and Distribution of Toxicogenomic Information. *Molecular Pharmacology*. 2005;67(4):1360-1368.
- Hultman, et al. Bidirectional Solid-Phase Sequencing of In Vitro-Amplified Plasmid DNA. *BioTechniques*. 1991;10(1):84-93.
- International Preliminary Report for corresponding PCT Application No. PCT/CA2000/01421 dated Feb. 14, 2002.
- International Preliminary Report for corresponding PCT Application No. PCT/US2005/018678 dated Nov. 13, 2007.
- International Preliminary Report for corresponding PCT Application No. PCT/US2005/033347 dated Mar. 20, 2007.
- International Preliminary Report for corresponding PCT Application No. PCT/US2007/007381 dated Sep. 23, 2008.
- International Preliminary Report for corresponding PCT Application No. PCT/US2007/02721 dated Aug. 5, 2008.
- International Preliminary Report for corresponding PCT Application No. PCT/US2007/061573 dated Aug. 26, 2008.
- International search report dated Oct. 6, 2010 for PCT Application No. US10/37545.
- International search report dated Apr. 5, 2001 for PCT Application No. CA2000/01421.
- International search report dated May 14, 2010 for PCT Application No. US2009/06640.
- International search report dated Jul. 11, 2008 for PCT Application No. US07/61573.
- International search report dated Jul. 30, 2010 for PCT Application No. US2010/36464.
- International search report dated Aug. 18, 2009 for PCT Application No. US09/00419.
- International search report dated Aug. 23, 2006 for PCT Application No. US2005/033347.
- International search report dated Aug. 26, 2004 PCT Application No. US2003/41466.
- International search report dated Sep. 25, 2007 for PCT Application No. US2007/02721.
- International Search Report for PCT/US2005/033347.
- Jacobson, et al. Electrokinetic Focusing in Microfabricated Channel Structures. *Anal. Chem.*, 1997, 69 (16), pp. 3212-3217.
- Japanese Office Action dated Dec. 21, 2010 for Application No. JP2001-540363 (in Japanese with English translation).
- Japanese Office Action dated Apr. 27, 2010 for Application No. JP2001-540363 (in Japanese with English translation).
- Ju, et al. Fluorescence energy transfer dye-labeled primers for DNA sequencing and analysis. *Proc. Natl. Acad. Sci. USA*. 1995;92:4347-4351.
- Kan, et al. A novel thermogelling matrix for microchannel DNA sequencing based on poly-N-alkoxyalkylacrylamide copolymers. *Electrophoresis*. 2003;24(24):4161-4169.
- Koh, et al. Integrating Polymerase Chain Reaction, Valving, and Electrophoresis in a Plastic Device for Bacterial Detection. *Analytical Chemistry*. 2003;75(17):4591-4598.
- Kopp, et al. Chemical Amplification Continuous-Flow PCR on a Chip. *Science*. 1998;280:1046-1048.
- Lagally, et al. Fully integrated PCR-capillary electrophoresis microsystem for DNA analysis. *Lab on a Chip*. 2001;1(2):102-107.
- Lagally, et al. Integrated Portable Genetic Analysis Microsystem for Pathogen/Infectious Disease Detection. *Analytical Chemistry*. 2004;76:3162-3170.
- Lagally, et al. Monolithic integrated microfluidic DNA amplification and capillary electrophoresis analysis system. *Sensors and Actuators*. 2000;B63(3):138-146.
- Lagally, et al. Single-Molecule DNA Amplification and Analysis in an Integrated Microfluidic Device. *Analytical Chemistry*. 2001;73(3):565-570.

- Lazar, et al. Subattomole-Sensitivity Microchip Nanoelectrospray Source with Time-of-Flight Mass Spectrometry Detection. *Analytical Chemistry*. 1999;71(17):3627-3631.
- Li, et al. Integration of Microfabricated Devices to Capillary Electrophoresis-Electrospray Mass Spectrometry Using a Low Dead Volume Connection: Application to Rapid Analyses of Proteolytic Digests. *Analytical Chemistry*. 1999;71(15):3036-3045.
- Li, et al. Rapid and sensitive separation of trace level protein digests using microfabricated devices coupled to a quadrupole—time-of-flight mass spectrometer. *Electrophoresis*. 2000;21:198-210.
- Li, et al. Separation and Identification of Peptides from Gel-Isolated Membrane Proteins Using a Microfabricated Device for Combined Capillary Electrophoresis/Nanoelectrospray Mass Spectrometry. *Analytical Chemistry*. 2000;72(3):599-609.
- Licklider, et al. A Micromachined Chip-Based Electrospray Source for Mass Spectrometry. *Analytical Chemistry*. 2000;72(2):367-375.
- Lisec, et al. A bistable pneumatic microswitch for driving fluidic components. *Sensors and Actuators*. 1996;A54:746-749.
- Liu, et al. Automated parallel DNA sequencing on multiple channel microchips. *Proc. Natl. Acad. Sci. USA*. 2000;97(10):5369-5374.
- Liu, et al. Optimization of High-Speed DNA Sequencing on Microfabricated Capillary Electrophoresis Channels. *Analytical Chemistry*. 1999;71:566-573.
- Margulies, et al. Genome sequencing in microfabricated high-density picolitre reactors. *Nature*. 2005;437(7057):376-80. (Abstract only).
- Melin, et al. A Passive 2-Dimensional Liquid Sample Micromixer. 7th International Conference on Miniaturized Chemical and Biochemical Analysis Systems. 2003;167170.
- MillGat pump user manual, version 2.12, published 2005, pp. 1-28.
- Mitra, et al. Digital genotyping and haplotyping with polymerase colonies. *Proc Natl Acad Sci USA*. 2003.100(10):15926-5931.
- Norris, et al. Fully-integrated, multiplexed STR-based human identification using a single microfluidic chip and automated instrument. Available at <http://www.promega.com/geneticidproc/ussymp20proc/oralpresentations/landersbienvenue.pdf>. Accessed Jun. 2, 2010.
- Obeid, et al. Microfabricated Device for DNA and RNA Amplification by Continuous-Flow Polymerase Chain Reaction and Reverse Transcription-Polymerase Chain Reaction with Cycle Number Selection. *Analytical Chemistry*. 2003;75(2): 288-295.
- Ocvirk, et al. High Performance Liquid Chromatography Partially Integrated onto a Silicon Chip. *Analytical Methods and Instrumentation*. 1995;2:74-82.
- Ocvirk, et al. Optimization of confocal epifluorescence microscopy for microchip-based miniaturized total analysis systems. *The Analyst*. 1998;123:1429-1434.
- Office Action Final dated Feb. 19, 2008 issued in U.S. Appl. No. 10/540,658.
- Office Action Final dated Feb. 6, 2008 issued in U.S. Appl. No. 11/139,018.
- Office Action mailed Apr. 27, 2007 in U.S. Appl. No. 11/139,018, filed May 25, 2005.
- Office Action mailed Jul. 2, 2007 in U.S. Appl. No. 10/540,658, filed Jun. 23, 2005.
- Office Action mailed Jul. 12, 2007 in U.S. Appl. No. 10/750,533, filed Dec. 29, 2003.
- Ohuri, et al. Partly disposable three-way microvalve for a medical micro total analysis system (muTAS). *Sensors and Actuators*. 1998;A64(1): 57-62.
- Oleschuk, et al. Trapping of Bead-Based Reagents within Microfluidic Systems: On-Chip Solid-Phase Extraction and Electrochromatography. *Analytical Chemistry*. 2000;72:585-590.
- Olsen, et al. Immobilization of DNA Hydrogel Plugs in Microfluidic Channels. *Analytical Chemistry*. 2002;74:1436-1441.
- Paegel, et al. High-throughput DNA sequencing with a 96-lane capillary array electrophoresis bioprocessor. *Proc Natl Acad Sci USA*. 2002;99:574-579.
- Paegel, et al. Microchip Bioprocessor for Integrated Nanovolume Sample Purification and DNA Sequencing. *Analytical Chemistry*. 2002;74(19):5092-5098.
- Paegel, et al. Microfluidic devices for DNA sequencing: sample preparation and electrophoretic analysis. *Current Opinion in Biotechnology*. 2003;14(1):42-50.
- Paegel, et al. Turn Geometry for Minimizing Band Broadening in Microfabricated Capillary Electrophoresis Channels. *Analytical Chemistry*. 2000;72:3030-3037.
- PCT Notification of Transmittal of the International Search Report and The Written Opinion of the International Searching Authority, or the Declaration, mailed Jun. 17, 2008, Application No. PCT/US2007/082568 (UCALP067WO).
- Peterson, et al. Enzymatic Microreactor-on-a-Chip: Protein Mapping Using Trypsin Immobilized on Porous Polymer Monoliths Molded in Channels of Microfluidic Devices. *Analytical Chemistry*. 2002;74:4081-4088.
- Ramsey, et al. Generating Electrospray from Microchip Devices Using Electroosmotic Pumping. *Analytical Chemistry*. 1997;69(6):1174-1178.
- Rohr, et al. Porous polymer monoliths: Simple and efficient mixers prepared by direct polymerization in the channels of microfluidic chips. *Electrophoresis*. 2001;22:3959-3967.
- Rye, et al. High-sensitivity two-color detection of double-stranded DNA with a confocal fluorescence gel scanner using ethidium homodimer and thiazole orange. *Nucleic Acids Research*. 1991;19(2):327-333.
- Scherer, et al. High-Pressure Gel Loader for Capillary Array Electrophoresis Microchannel Plates. *Biotechniques*. 2001;31(5):1150-1154.
- Schomburg, et al. Design Optimization of Bistable Microdiaphragm Valves. *Sensors and Actuators*. 1998;A64:259-264.
- Seifar, et al. Capillary electrochromatography with 1.8- μ m ODS-modified porous silica particles. *Journal of Chromatography*. 1998; A808:71-77.
- Simpson, et al. High-throughput genetic analysis using microfabricated 96-sample capillary array electrophoresis microplates. *Proc Natl Acad Sci USA*. 1998;95:2256-2261.
- Simpson, et al. Microfabrication Technology for the Production of Capillary Array Electrophoresis Chips. *Biomedical Microdevices*. 1998;1:7-26.
- Soper, et al. Sanger DNA Sequencing Reactions Performed in a Solid-Phase Nanoreactor Directly Coupled to Capillary Gel Electrophoresis. *Analytical Chemistry*. 1998;70:4036-4043.
- Spiering, et al. Novel microstructures and technologies applied in chemical analysis techniques. 1997 International Conference on Solid-State Sensors and Actuators. 1997;1:511-514.
- Takao, et al. A Pneumatically Actuated Full In-Channel Microvalve With MOSFET-Like Function in Fluid Channel Networks. *Journal of Microelectromechanical Systems*. 2002;11(5):421-426.
- Takao, et al. Microfluidic Integrated Circuits for Signal Processing Using Analogous Relationship Between Pneumatic Microvalve and MOSFET. *Journal of Microelectromechanical Systems*. 2003;12(4):497-505.
- Thomas, et al. Application of Genomics to Toxicology Research. *Environmental Health Perspectives*. 2002;110(6):919-923.
- Thorsen, et al. Microfluidic Large-Scale Integration. *Science*. 2002;298(5593):580-584.
- Tice, et al. Formation of Droplets and Mixing in Multiphase Microfluidics at Low Values of the Reynolds and the Capillary Numbers. *Langmuir*. 2003;19:9127-9133.
- Unger, et al. Monolithic Microfabricated Valves and Pumps by Multilayer Soft Lithography. *Science*. 2000;288:113-116.
- Van Der Moolen, et al. A Micromachined Injection Device for CZE: Application to Correlation CZE. *Analytical Chemistry*. 1997;69(20):4220-4225.
- Van Der Moolen, et al. Correlation Capillary Zone Electrophoresis, a Novel Technique to Decrease Detection Limits. *Chromatographia*. 1995;40(7/8):368-374.
- Vazquez, et al. Electrophoretic Injection within Microdevices. *Analytical Chemistry*. 2002;74:1952-1961.
- Veenstra, et al. The design of an in-plane compliance structure for microfluidical systems. *Sensors and Actuators*. 2002;B81:377-383.
- Waller, et al. Quantitative Immunocapture PCR Assay for Detection of *Campylobacter jejuni* in Foods. *Applied Environmental Microbiology*. 2000; 66(9):4115-4118.

- Weimer, et al. Solid-Phase Capture of Proteins, Spores, and Bacteria. *Applied Environmental Microbiology*. 2001;67(3):1300-1307.
- Wen, et al. Microfabricated isoelectric focusing device for direct electrospray ionization-mass spectrometry. *Electrophoresis*. 2000;21:191-197.
- Wikipedia brochure for defining stochastic process. Sep. 2, 2009.
- Williams, et al. Amplification of complex gene libraries by emulsion PCR. *Nature Methods*. 2006;3(7):545-50.
- Woolley, et al. Functional Integration of PCR Amplification and Capillary Electrophoresis in a Microfabricated DNA Analysis Device. *Analytical Chemistry*. 1996;68(23):4081-4086.
- Wright, et al. Behavior and Use of Nonaqueous Media without Supporting Electrolyte in Capillary Electrophoresis and Capillary Electrochromatography. *Analytical Chemistry*. 1997;69(16):3251-3259.
- Xiang, et al. An Integrated Microfabricated Device for Dual Microdialysis and On-Line ESI-Ion Trap Mass Spectrometry for Analysis of Complex Biological Samples. *Analytical Chemistry*. 1999;71(8):1485-1490.
- Xue, et al. Integrated Multichannel Microchip Electrospray Ionization Mass Spectrometry: Analysis of Peptides from On-Chip Tryptic Digestion of Melittin. *Rapid Communications in Mass Spectrometry*. 1997;11:1253-1256.
- Xue, et al. Multichannel Microchip Electrospray Mass Spectrometry. *Analytical Chemistry*. 1997;69(3):426-430.
- Yang, et al. A MEMS thermopneumatic silicone rubber membrane valve. *Sensors and Actuators*. 1998;A64(1):101-108.
- Yu, et al. Preparation of Monolithic Polymers with Controlled Porous Properties for Microfluidic Chip Applications Using Photoinitiated Free Radial Polymerization. *Journal of Polymer Science*. 2002;40:755-769.
- Yu, et al. Towards stationary phases for chromatography on a microchip: Molded porous polymer monoliths prepared in capillaries by photoinitiated in situ polymerization as separation media for electrochromatography. *Electrophoresis*. 2000;21:120-127.
- Zhang, et al. A Microdevice with Integrated Liquid Junction for Facile Peptide and Protein Analysis by Capillary Electrophoresis/Electrospray Mass Spectrometry. *Analytical Chemistry*. 2000;72(5):1015-1022.
- Zhang, et al. Microfabricated Devices for Capillary Electrophoresis-Electrospray Mass Spectrometry. *Analytical Chemistry*. 1999;71(15):3258-3264.
- U.S. Appl. No. 13/075,165, filed Mar. 29, 2011, Eberhart et al.
- European search report and search opinion dated Jun. 6, 2011 for Application No. 10011511.2.
- International search report and written opinion dated Mar. 24, 2011 for PCT Application No. US2010/58227.
- International search report and written opinion dated Jun. 9, 2011 for PCT Application No. US2011/30973.
- Notice of allowance dated Jun. 9, 2011 for U.S. Appl. No. 12/831,949.
- U.S. Appl. No. 13/202,877, filed Aug. 23, 2011, Vangbo et al.
- U.S. Appl. No. 13/202,884, filed Aug. 23, 2011, Jovanovich et al.
- Chinese office action dated Jan. 31, 2011 for CN 200580035911.7. (In Chinese with English translation).
- U.S. Appl. No. 13/113,968, filed May 23, 2011, Majlof et al.
- Armani, et al. Re-configurable fluid circuits by PDMS elastomer micromachining. *Proceedings of IEEE Micro Electro Mechanical Systems: MEMS*. 1999; 222-227.
- Bennett, et al. Toward the 1,000 dollars human genome. *Pharmacogenomics*, 6 (4) 373-382. (Jun. 2005).
- Erratum for Margulies, et al. Genome sequencing in microfabricated high-density picolitre reactors. *Nature*. 2005;437(7057):376-80.
- Margulies, et al. *Nature*. 441(7089):120. (May 4, 2006).
- International search report dated Sep. 1, 2010 for PCT/US2010/040490.
- JAPIO Abstract of Arai (JP408327594A).*
- CAPLUS abstract of Krokhnin et al. ("Modified silica as a stationary phase for ion chromatography", *J. Chromatogr., A* (1995), 706(1+2), 93-8).*
- Ocvirk, Gregor et al., "High Performance Liquid Chromatography Partially Integrated onto a Silicon Chip," *Research Articles*, Nov. 1994, 9 pp.
- Spiering, Vincent L., et al., "Novel Microstructures and Technologies Applied in Chemical Analysis Techniques," *Transducers '97*, Jun. 1997, pp. 511-514.
- Ocvirk, Gregor et al., "Optimization of Confocal Epifluorescence Microscopy for Microchip-Based Miniaturized Total Analysis Systems," *The Analyst*, vol. 123, Jun. 1998, pp. 1429-1434.
- van der Moolen, J.N. et al., "Correlation Capillary Zone Electrophoresis, a Novel Technique to Decrease Detection Limits," *Chromatographia*, vol. 40, No. 7/8, Apr. 1995, pp. 368-374.
- van der Moolen, Johannes N. et al., "A Micromachined Injection Device for CZE: Application to Correlation CZE," *Analytical Chemistry*, vol. 69, No. 20, Oct. 1997, pp. 4220-4225.
- Kopp, Martin U. et al., "Chemical Amplification: Continuous-Flow PCR on a Chip," *Science*, vol. 280, May 1998, pp. 1046-1048.
- Xue Qifeng et al., "Multichannel Microchip Electrospray Mass Spectrometry," *Analytical Chemistry*, vol. 69, No. 3, Feb. 1997, pp. 426-430.
- Zhang, Bailin et al., "A Microdevice with Integrated Liquid Junction for Facile Peptide and Protein Analysis in Capillary Electrophoresis/Electrospray Mass Spectrometry," *Analytical Chemistry*, vol. 72, No. 5, Mar. 2000, pp. 1015-1022.
- Zhang, B. et al., "Microfabricated Devices for Capillary Electrophoresis-Electrospray Mass Spectrometry," *Analytical Chemistry*, vol. 71, No. 15, Aug. 1999, pp. 3258-3264.
- Licklider, Larry et al., "A Micromachined Chip-Based Electrospray Source for Mass Spectrometry," *Analytical Chemistry*, vol. 72, No. 2, Jan. 2000, pp. 367-375.
- Figeys, Daniel et al., "A Microfabricated Device for Rapid Protein Identification by Microelectrospray Ion Trap Spectrometry," *Analytical Chemistry*, vol. 69, No. 16, Aug. 1997, pp. 3153-3160.
- Figeys, Daniel et al., "An Integrated Microfluidics-Tandem Mass Spectrometry System for Automated Protein Analysis," *Analytical Chemistry*, vol. 70, No. 18, Sep. 1998, pp. 3728-3734.
- Figeys, Daniel et al., "Nanoflow Solvent Gradient Delivery from a Microfabricated Device for Protein Identifications by Electrospray Ionization Mass Spectrometry," *Analytical Chemistry*, vol. 70, No. 18, Sep. 1998, pp. 3721-3727.
- Chan, Jason H. et al., "Microfabricated Polymer Devices for Automated Sample Delivery of Peptides for Analysis by Electrospray Ionization Tandem Mass Spectrometry," *Analytical Chemistry*, vol. 71, No. 20, Oct. 1999, pp. 4437-4444.
- Figeys, Daniel et al., "Microfabricated Device Coupled with an Electrospray Ionization Quadrupole Time-of-Flight Mass Spectrometer: Protein Identifications Based on Enhanced-Resolution Mass Spectrometry and Tandem Mass Spectrometry Data," *Rapid Communications In Mass Spectrometry*, vol. 12, Aug. 1998, pp. 1435-1444.
- Xiang, Fan et al., "An Integrated Microfabricated Device for Dual Microdialysis and On-Line ESI-Ion Trap Mass Spectrometry for Analysis of Complex Biological Samples," *Analytical Chemistry*, vol. 71, No. 8, Apr. 1999, pp. 1485-1490.
- Wen, Jenny et al., "Microfabricated Isoelectric Focusing Device for Direct Electrospray Ionization-Mass Spectrometry," *Electrophoresis*, vol. 21, 2000, pp. 191-197.
- Lazar, Julia M. et al., "Subattomole-Sensitivity Microchip-Nanoelectrospray Source with Time-of-Flight Mass Spectrometry Detection," *Analytical Chemistry*, vol. 71, No. 17, Sep. 1999, p. 3627-3631.
- Ramsey, R.S. et al., "Generating Electrospray from Microchip Devices Using Electroosmotic Pumping," *Analytical Chemistry*, vol. 69, No. 6, Mar. 1997, pp. 1174-1178.
- Li, Jianjun et al., "Separation and Identification of Peptides from Gel-Isolated Membrane Proteins Using a Microfabricated Device for Combined Capillary Electrophoresis/Nanoelectrospray Mass Spectrometry," *Analytical Chemistry*, vol. 72, No. 3, Feb. 1999, pp. 599-609.
- Bings, Nicolas H. et al., "Microfluidic Devices Connected to Fused-Silica Capillaries with Minimal Dead Volume," *Analytical Chemistry*, vol. 71, No. 15, Aug. 1999, pp. 3292-3296.

Li, Jianjun et al., "Integration of Microfabricated Devices to Capillary Electrophoresis-Electrospray Mass Spectrometry Using a Low Dead Volume Connection: Application to Rapid Analyses of Proteolytic Digests," *Analytical Chemistry*, vol. 71, No. 15, Aug. 1999, pp. 3036-3045.

Li, Jianjun et al., "Rapid and Sensitive Separation of Trace Level Protein Digests Using Microfabricated Devices Coupled to a

Quadrupole—Time-of-Flight Mass Spectrometer," *Electrophoresis*, 2000, pp. 198-210.

Xue, Qifeng et al., "Integrated Multichannel Microchip Electrospray Ionization Mass Spectrometry: Analysis of Peptides from On Chip Tryptic Digestion of Melittin," *Rapid Communications in Mass Spectrometry*, vol. 11, Jun. 1997, pp. 1253-1256.

* cited by examiner

FIG. 1A

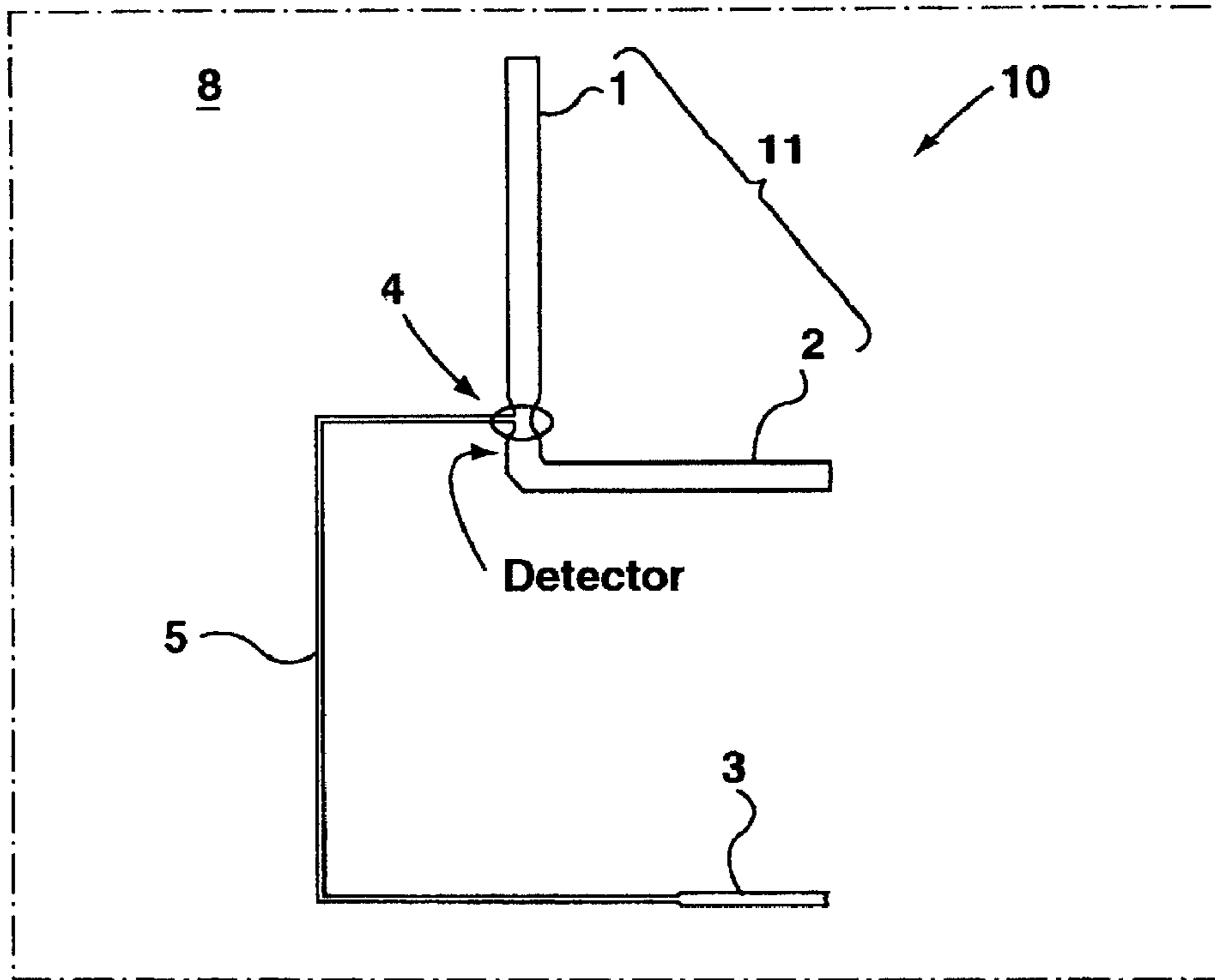
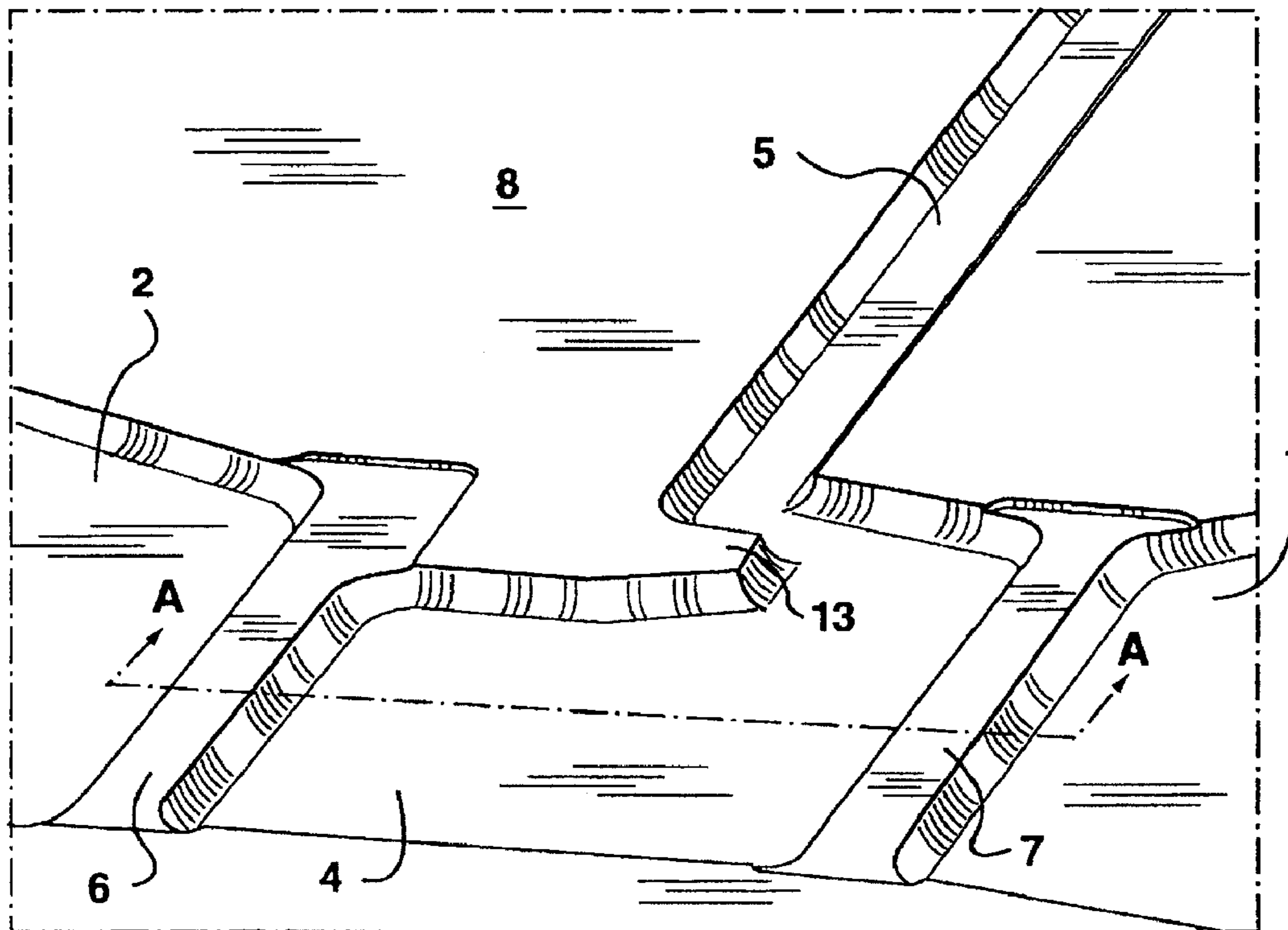


FIG. 1B



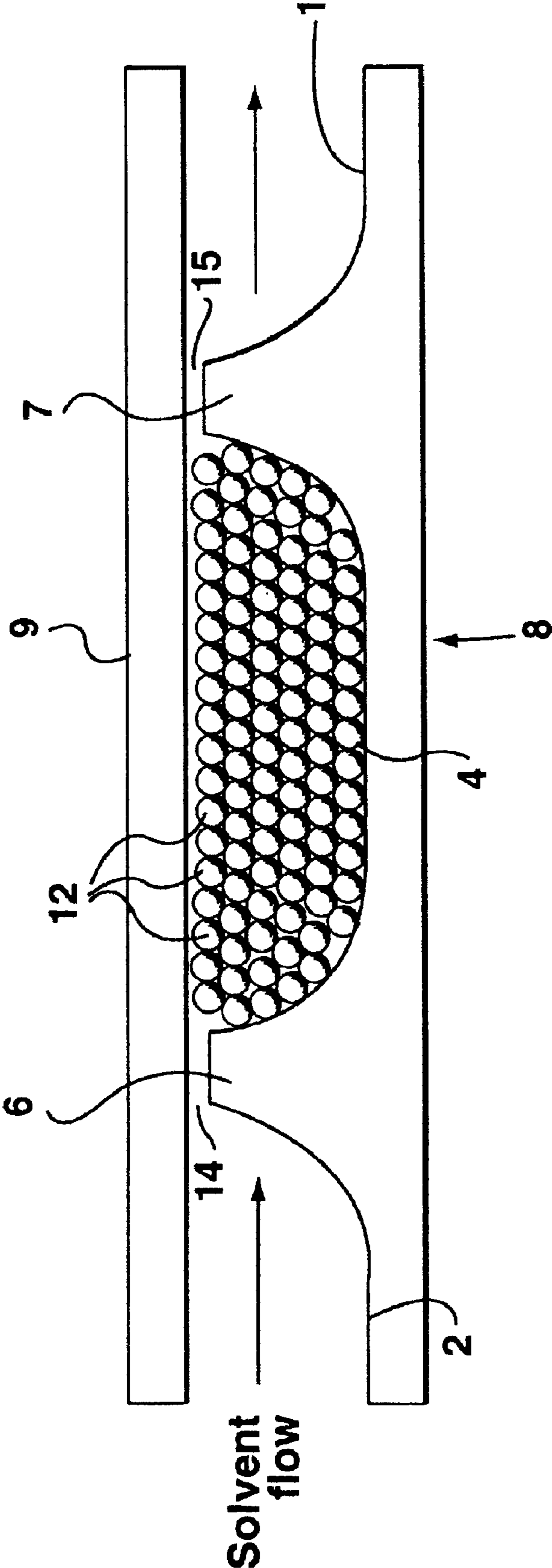


FIG. 2A

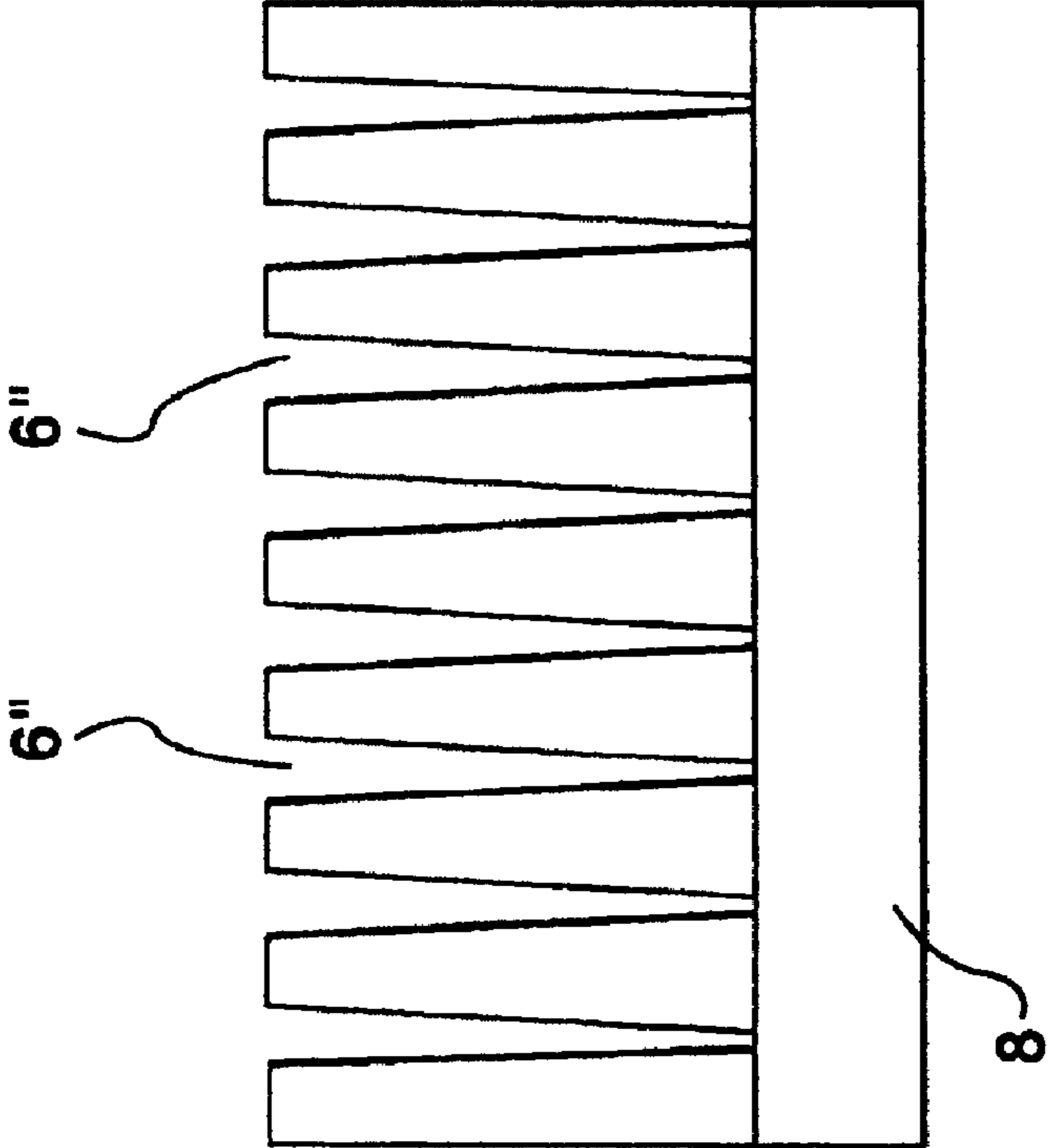


FIG. 2C

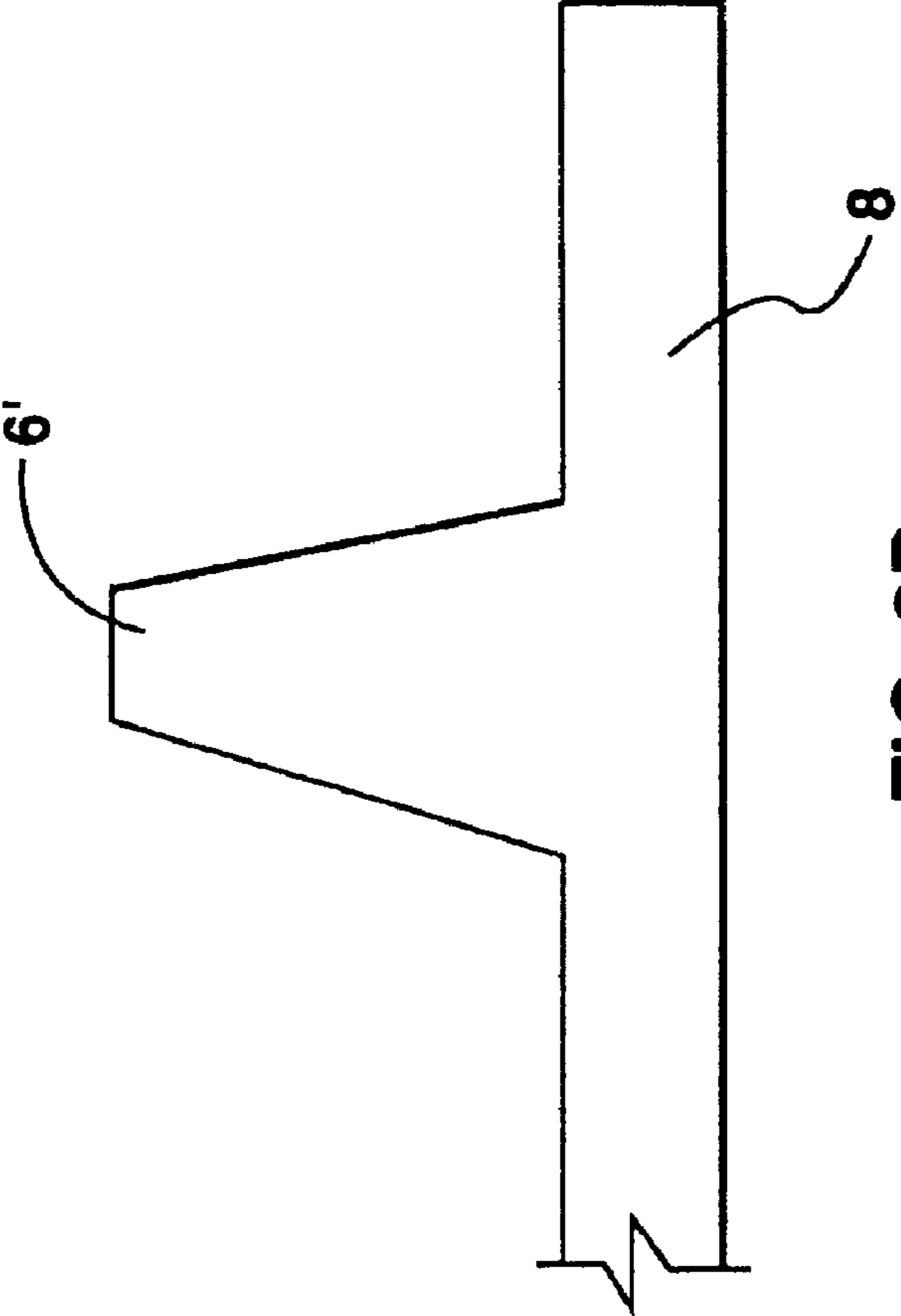


FIG. 2B

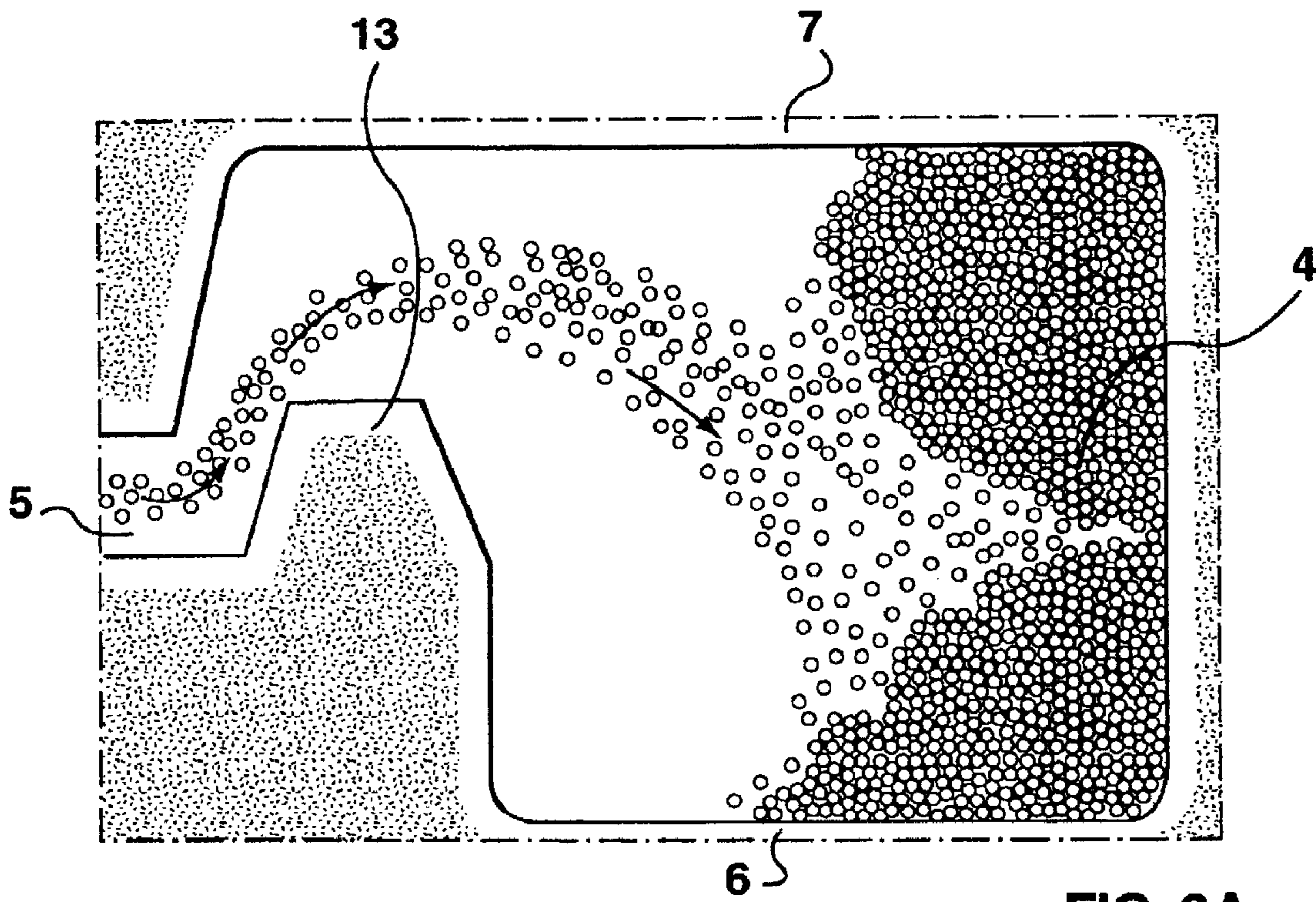


FIG. 3A

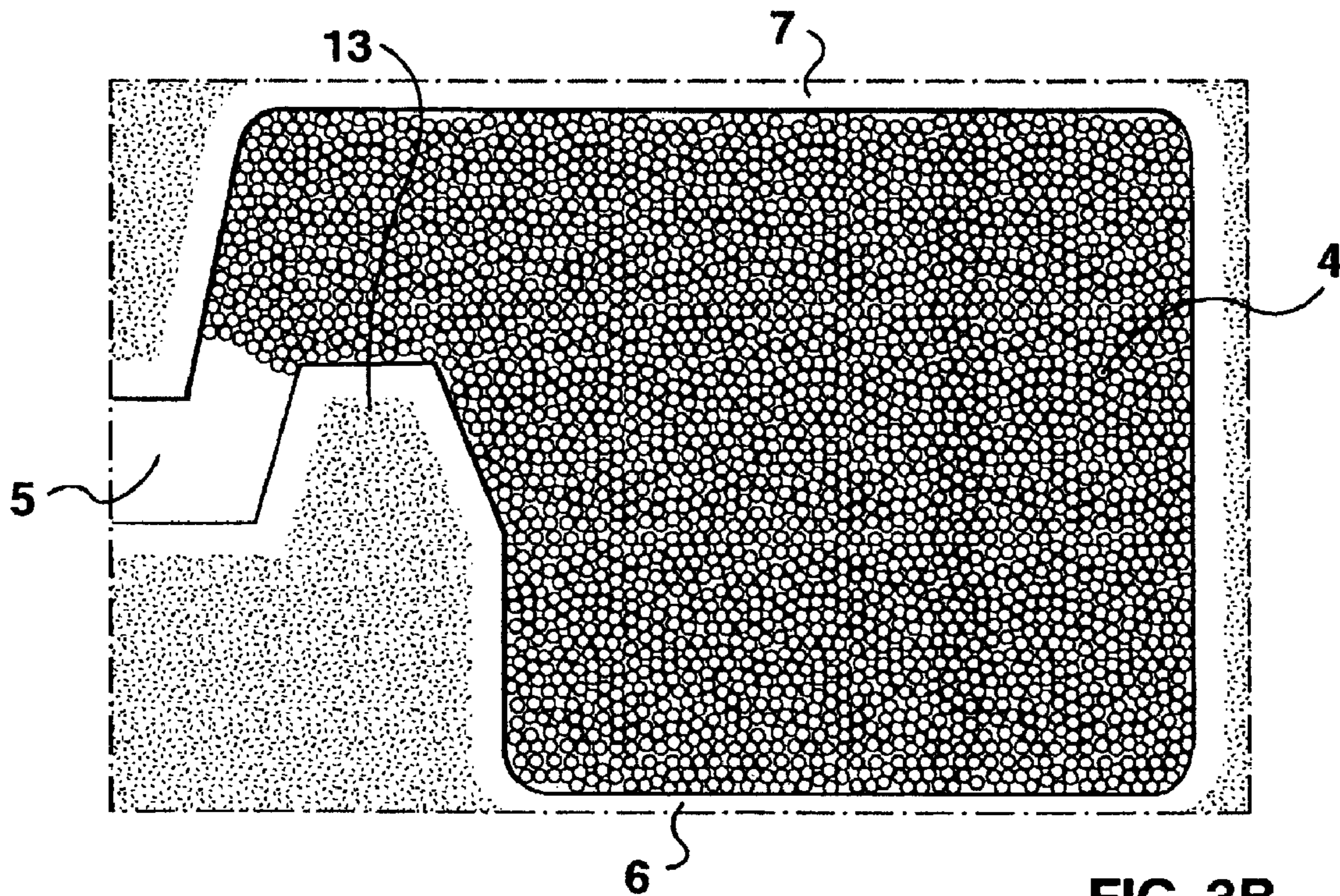


FIG. 3B

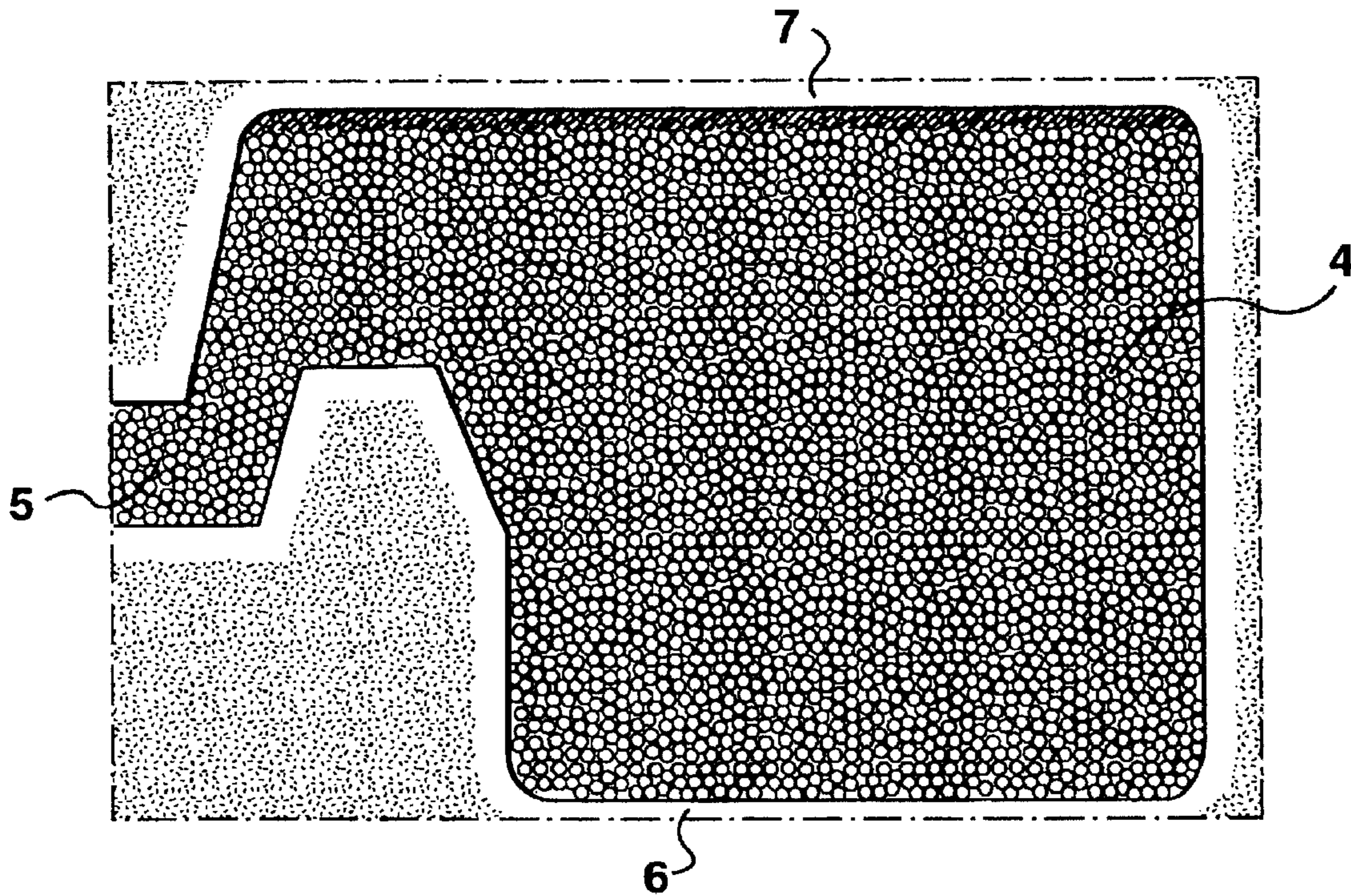


FIG. 4A

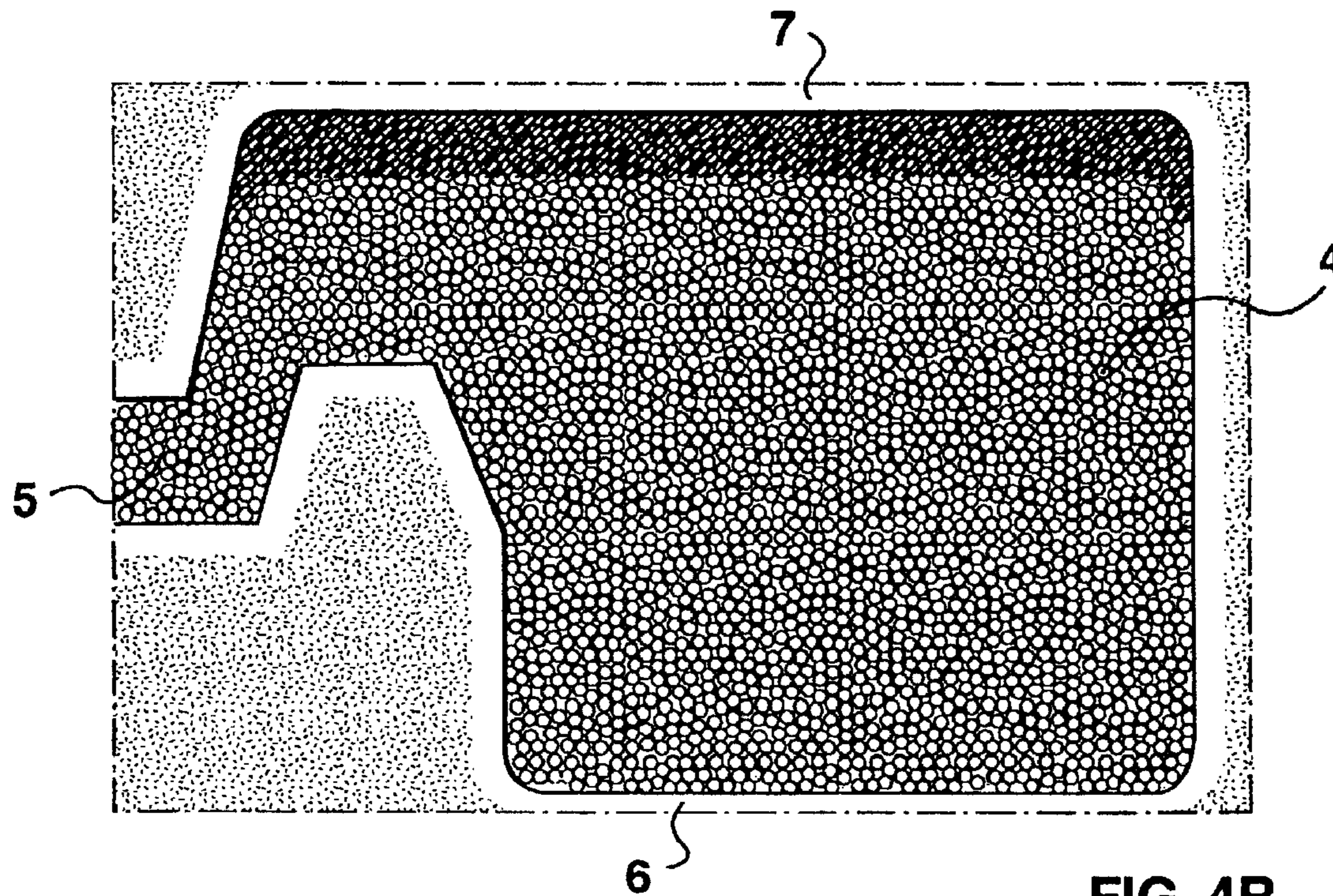


FIG. 4B

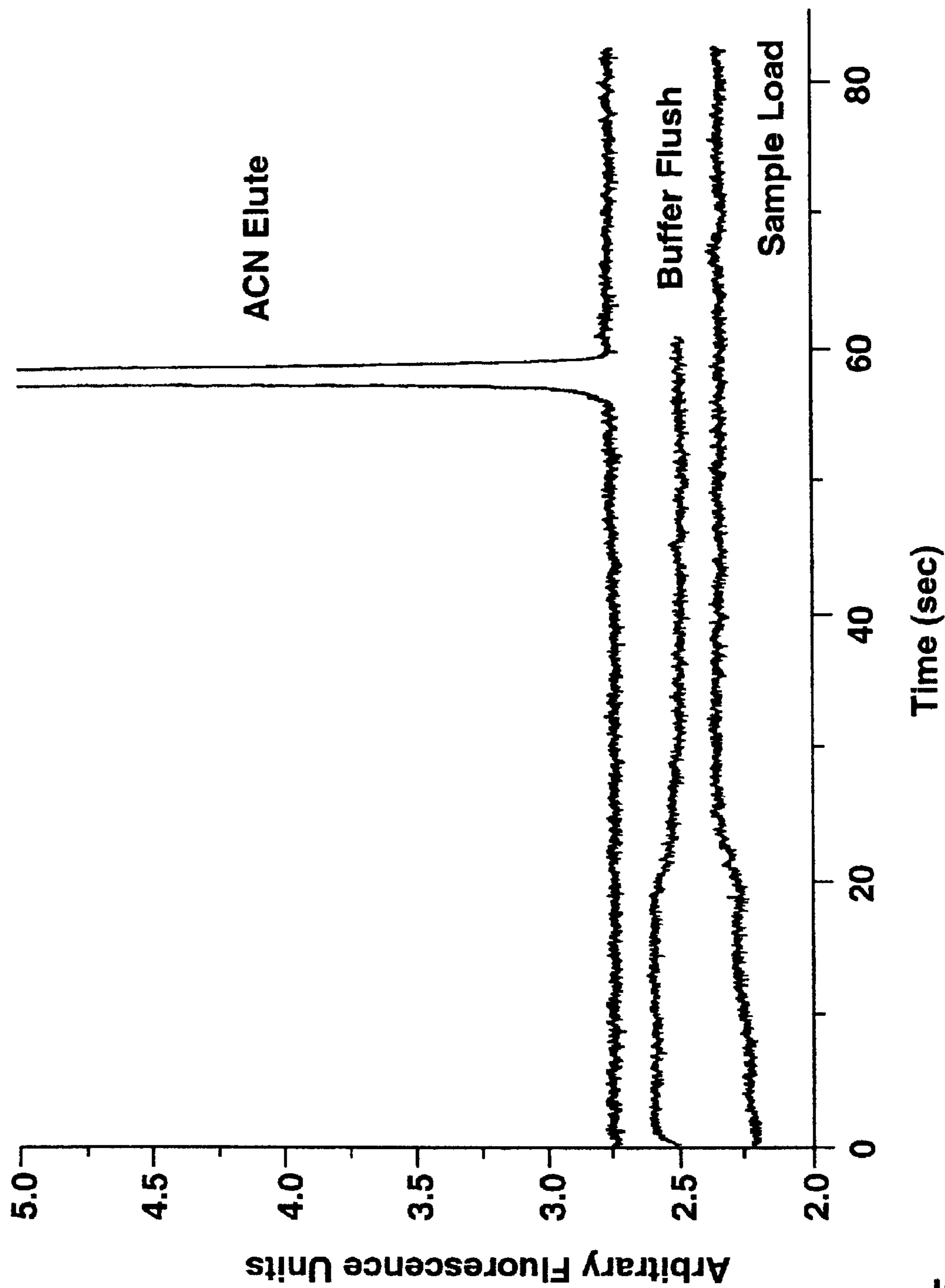


FIG. 5

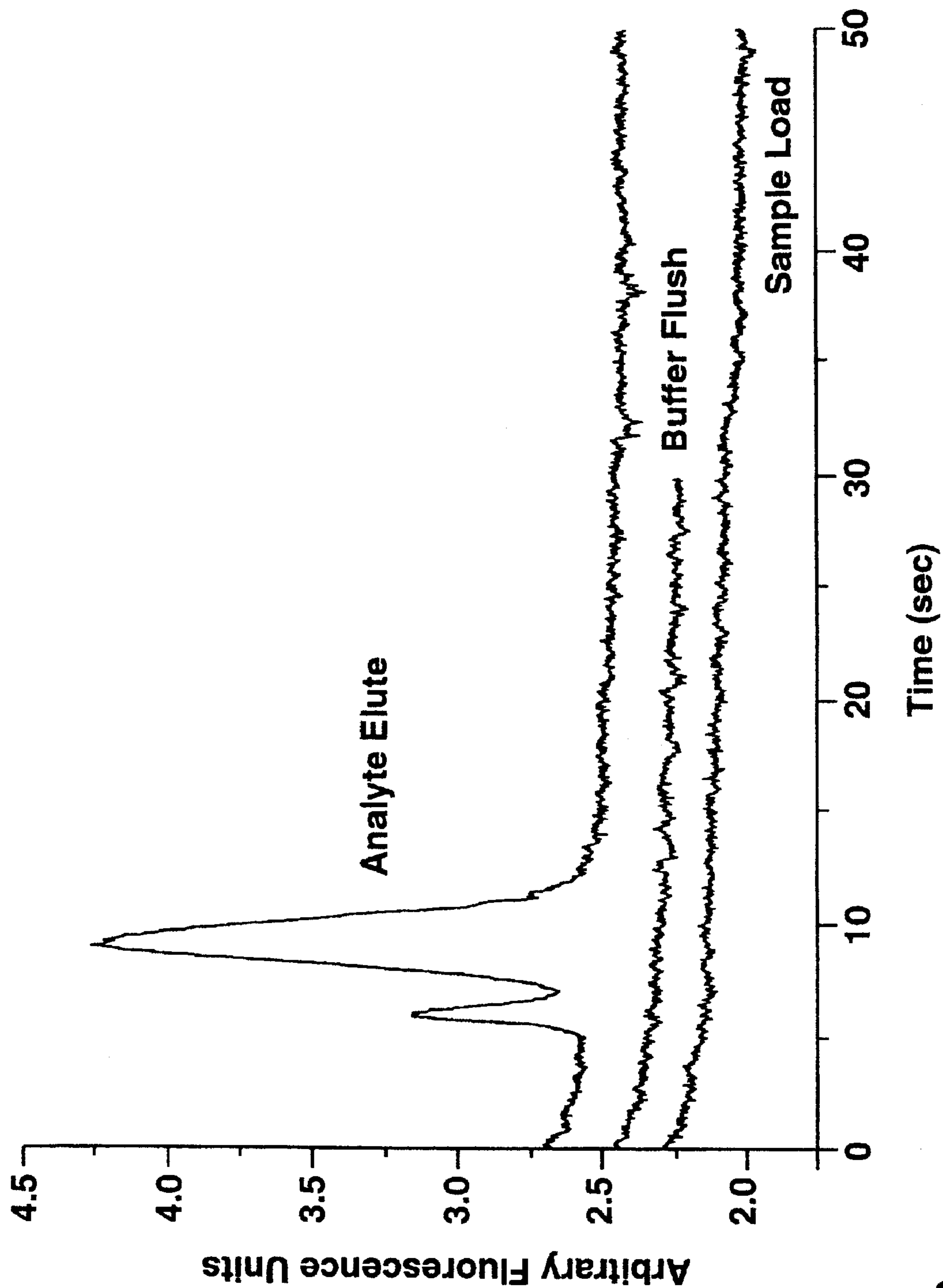
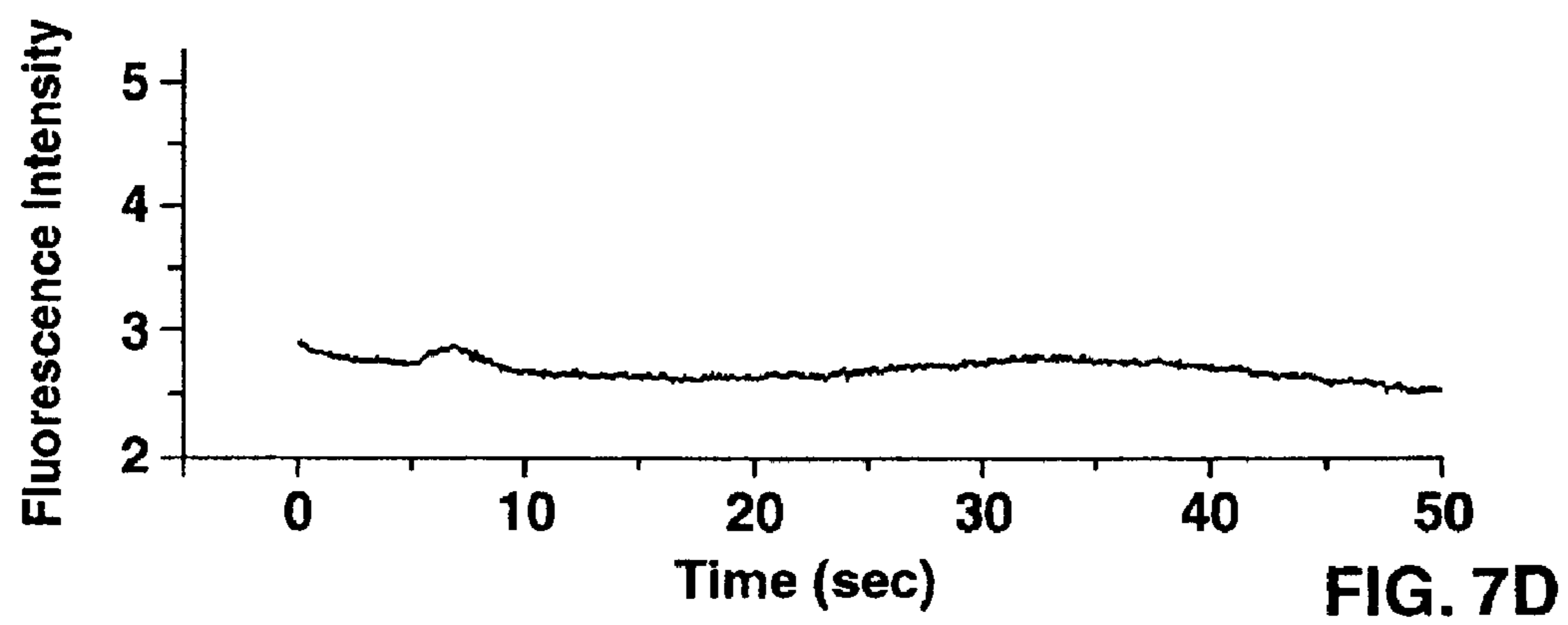
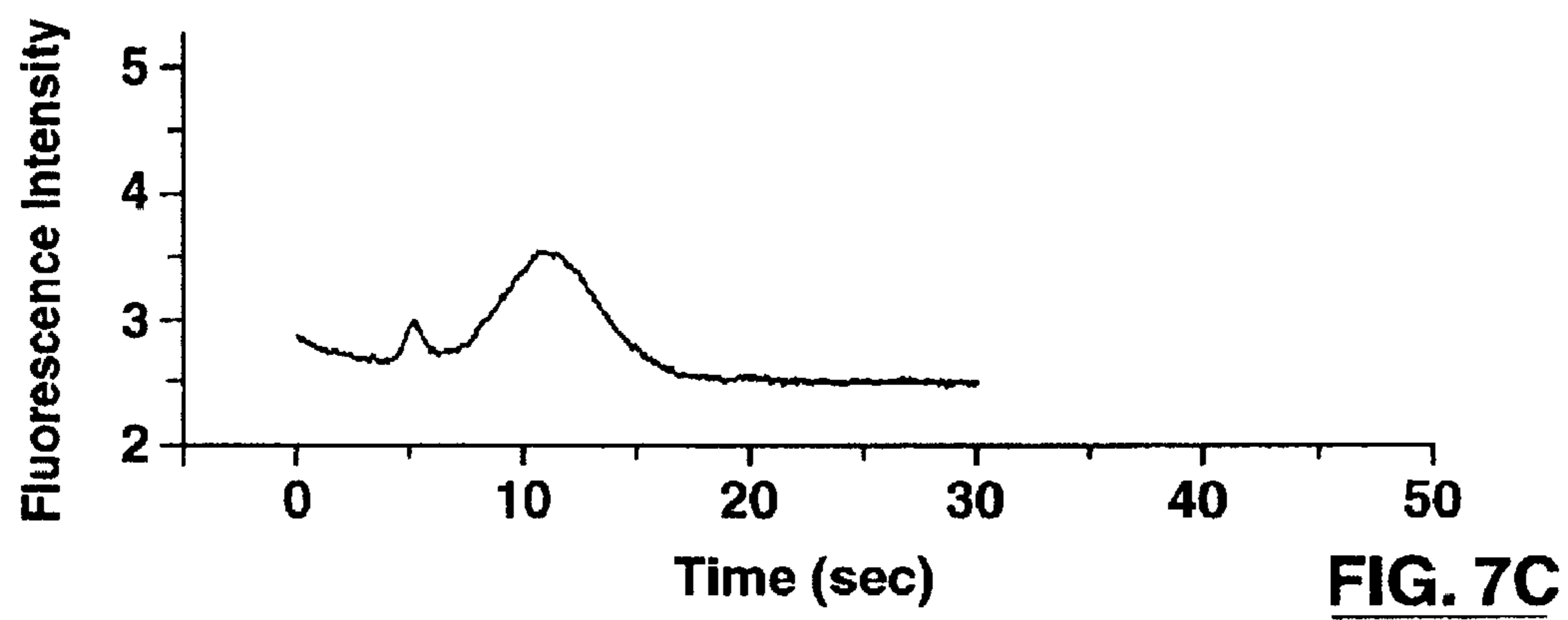
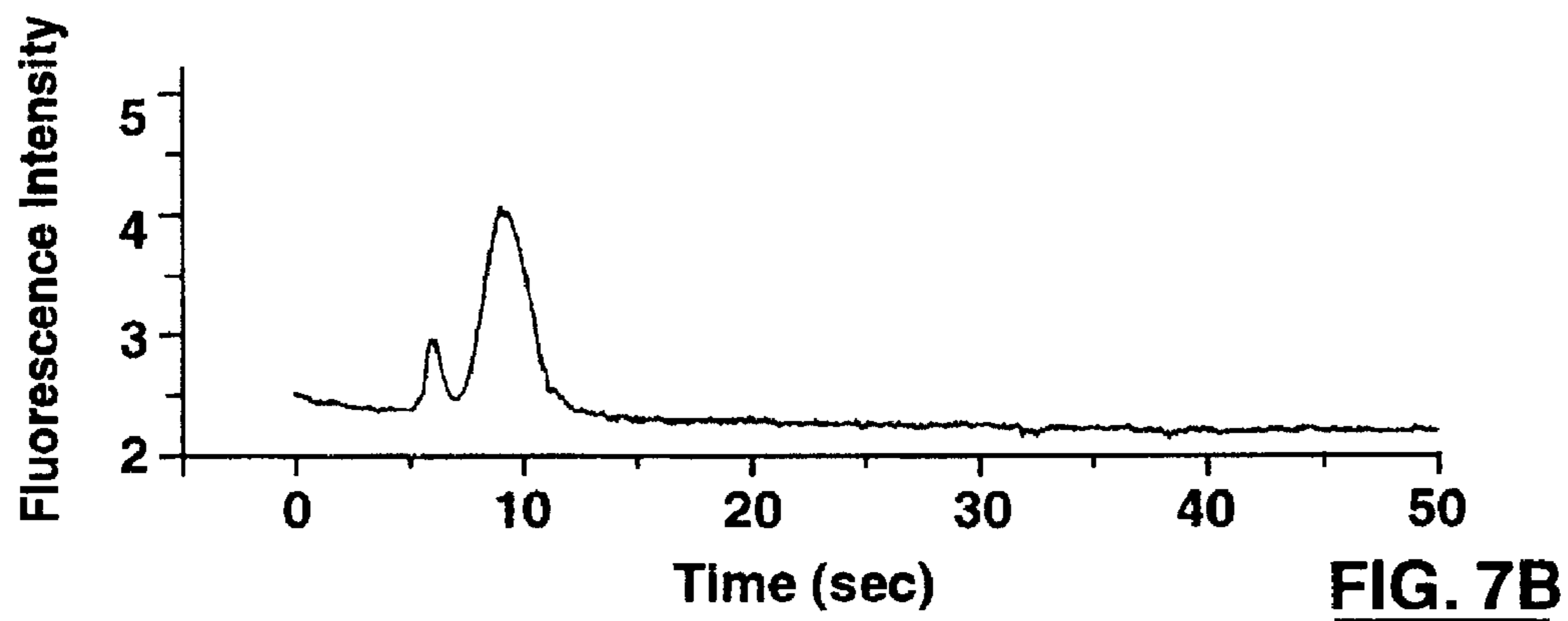
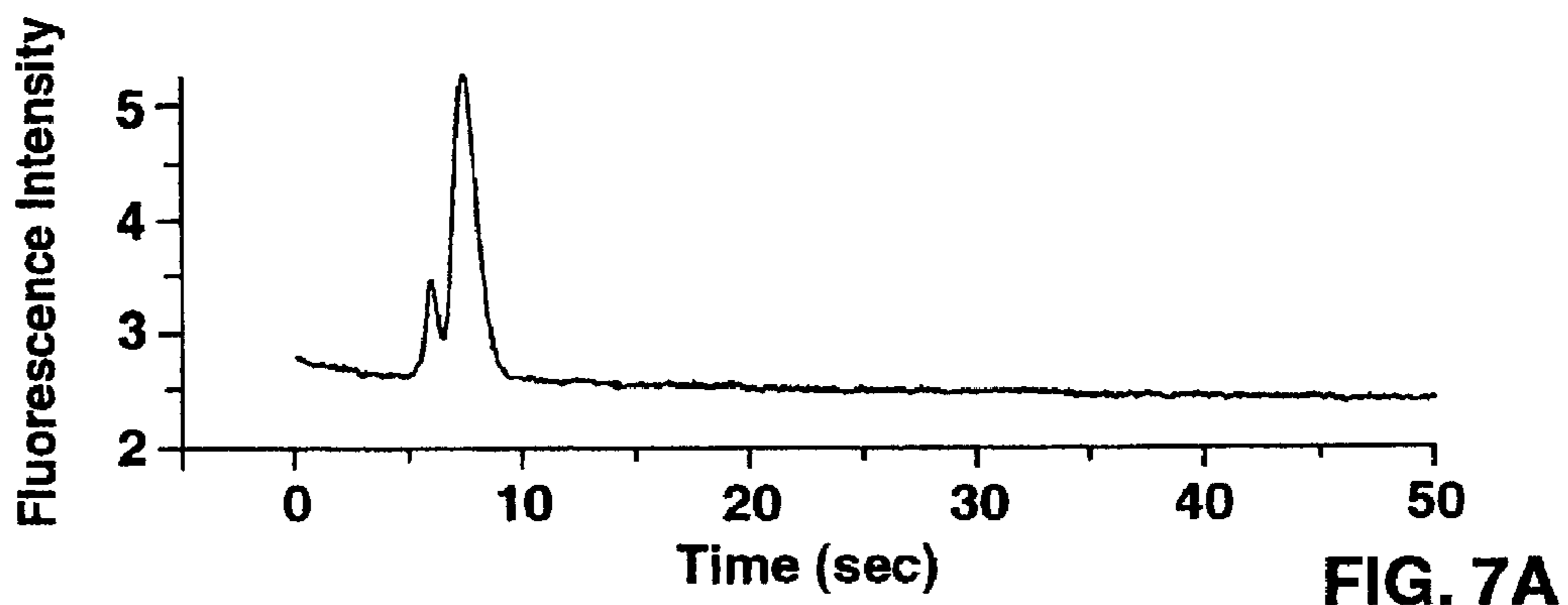


FIG. 6



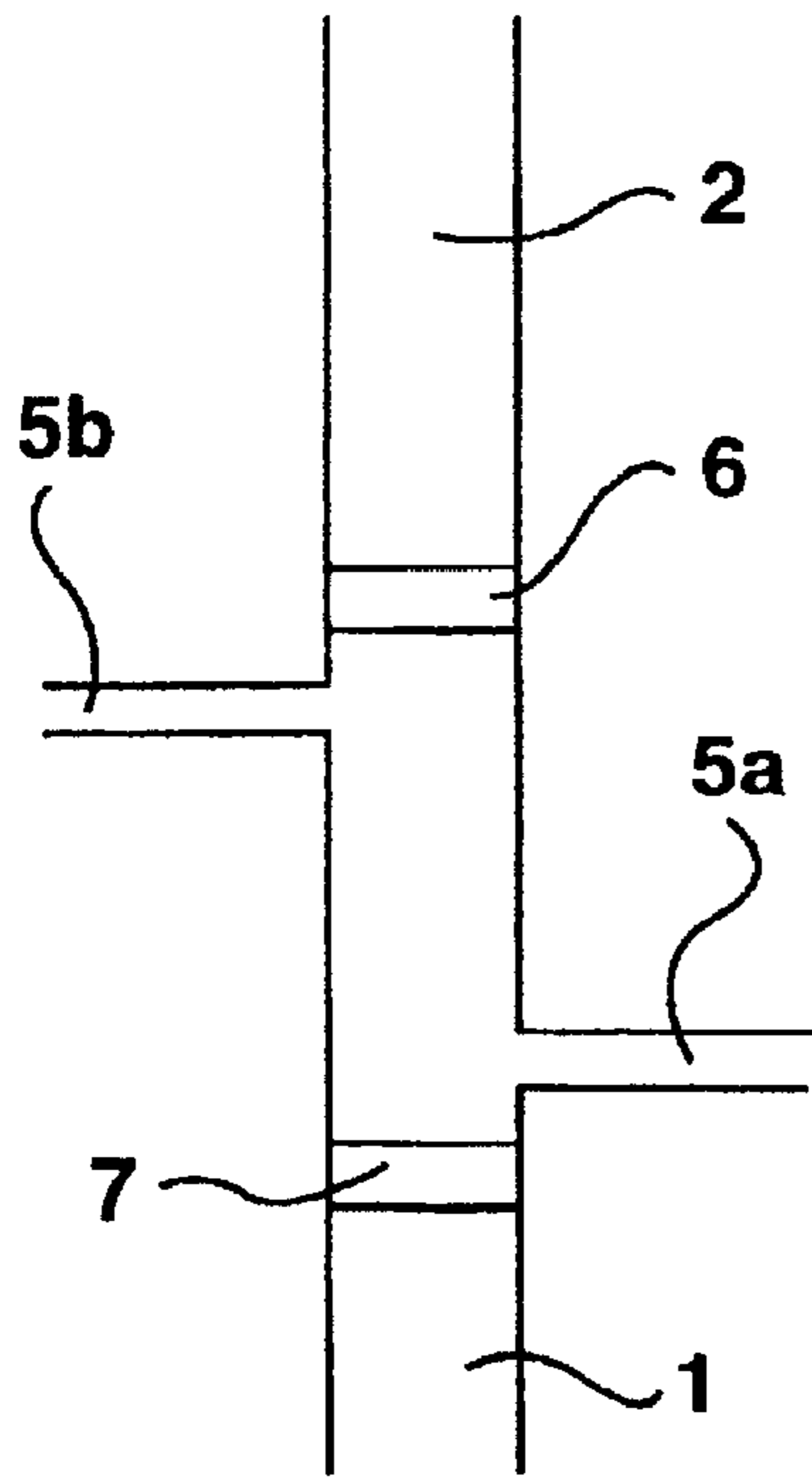


FIG. 8A

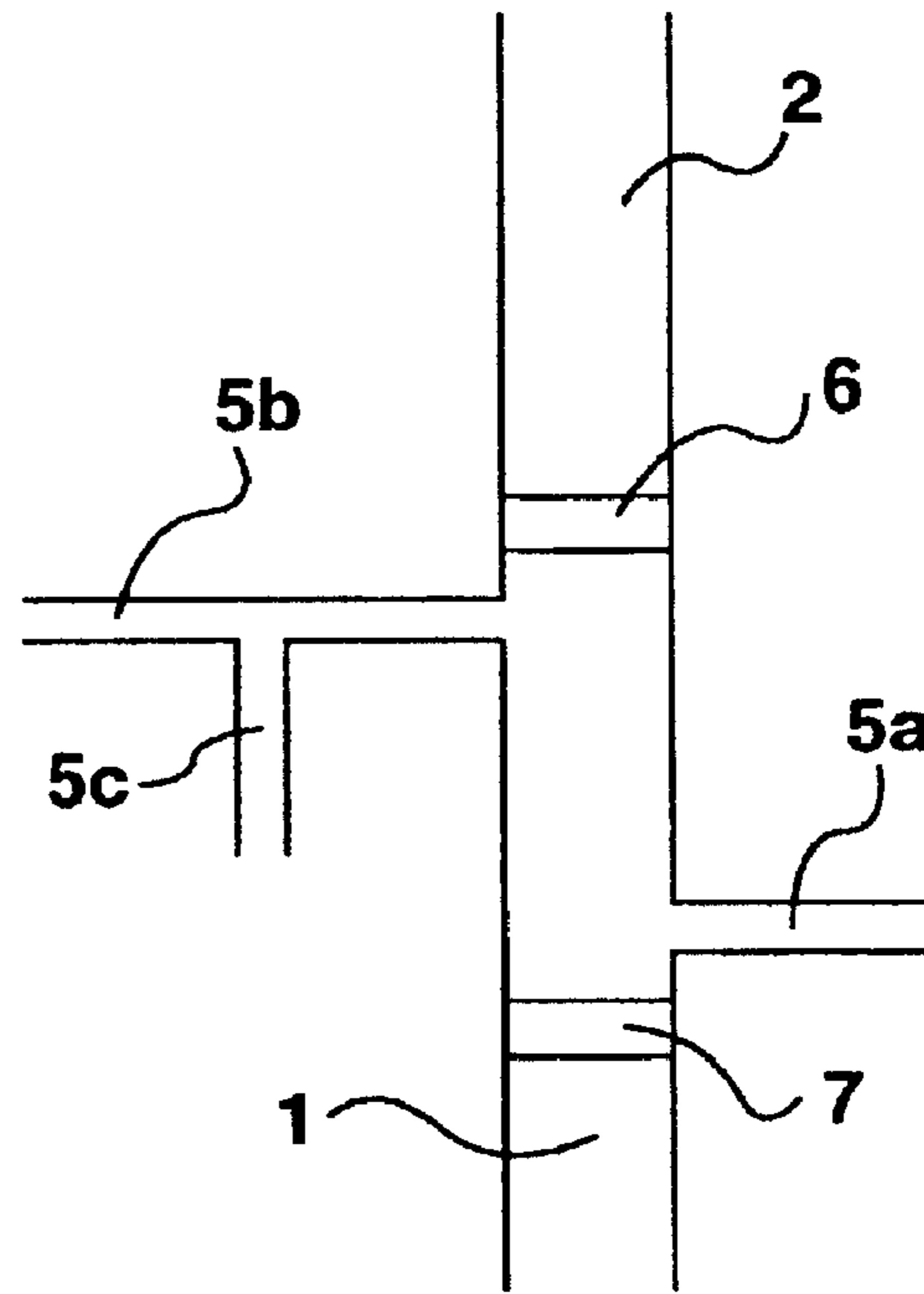


FIG. 8B

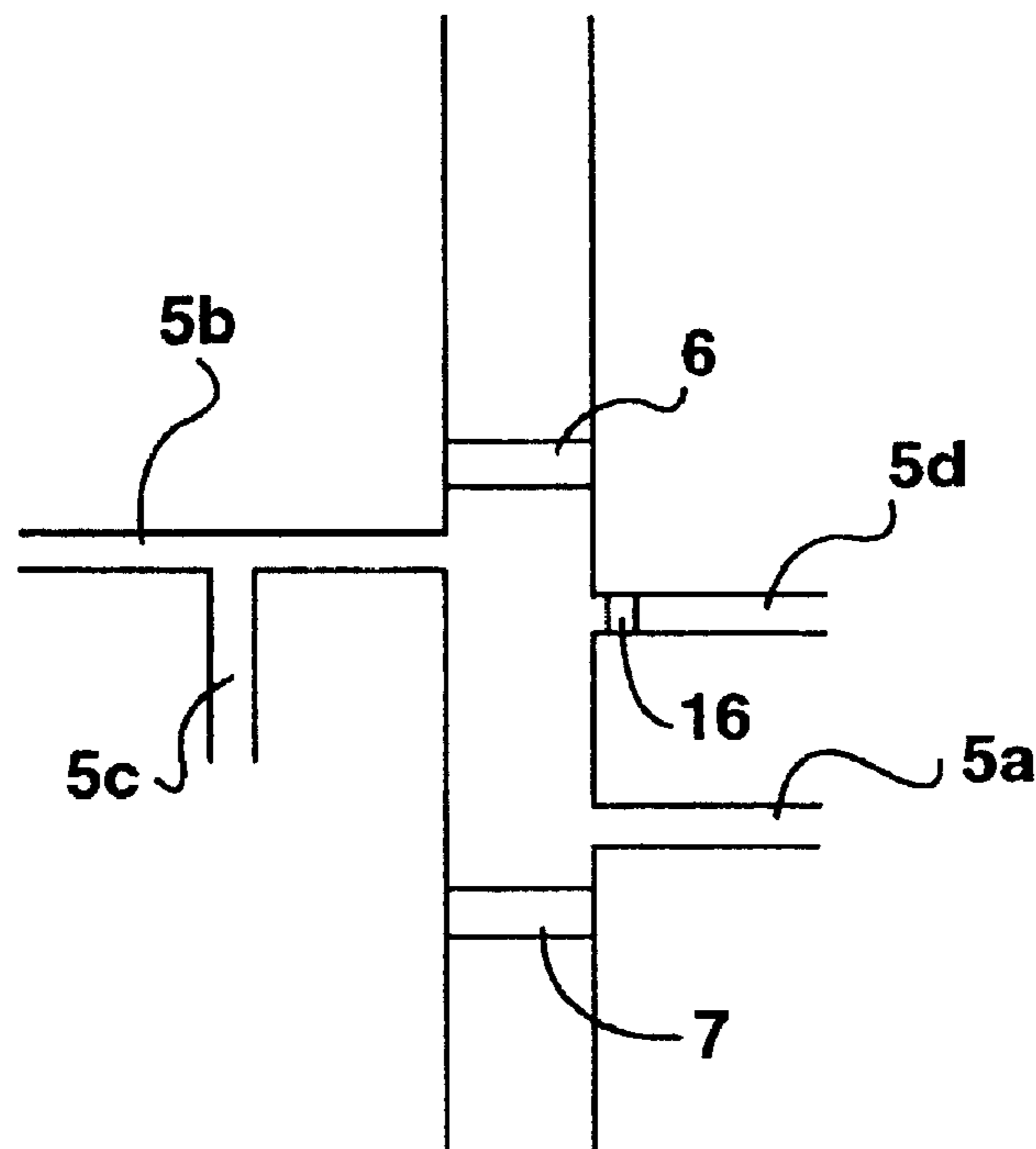


FIG. 8C

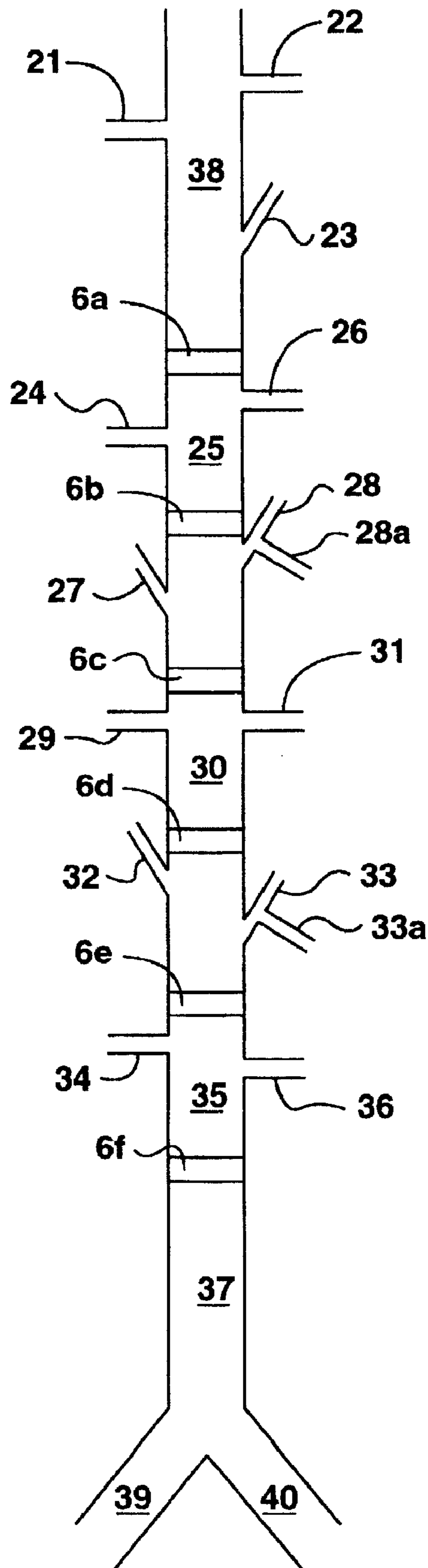


FIG. 9

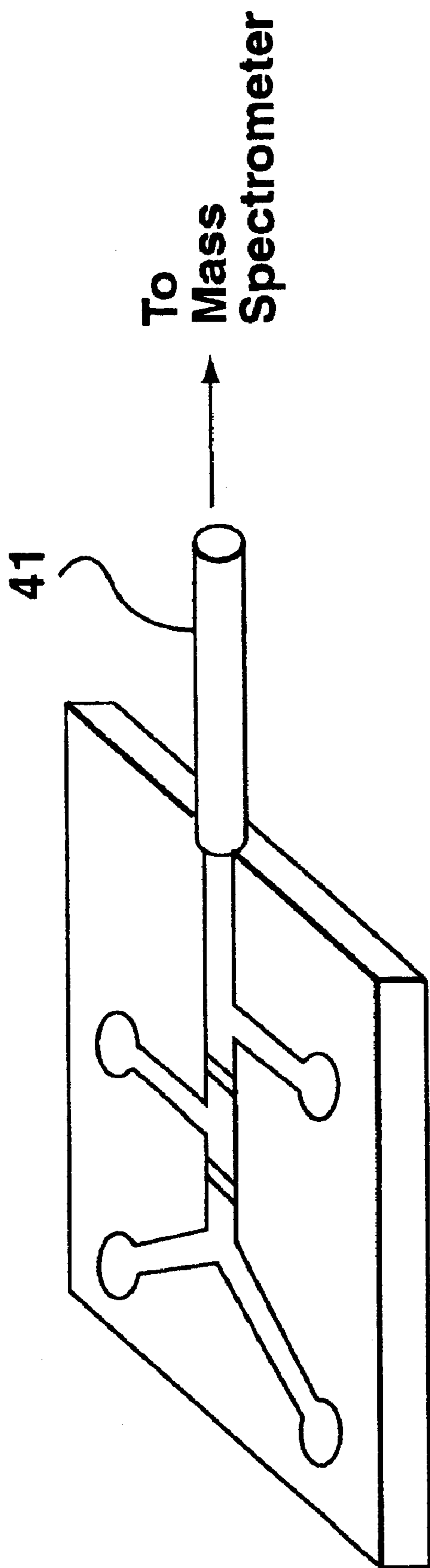


FIG. 10

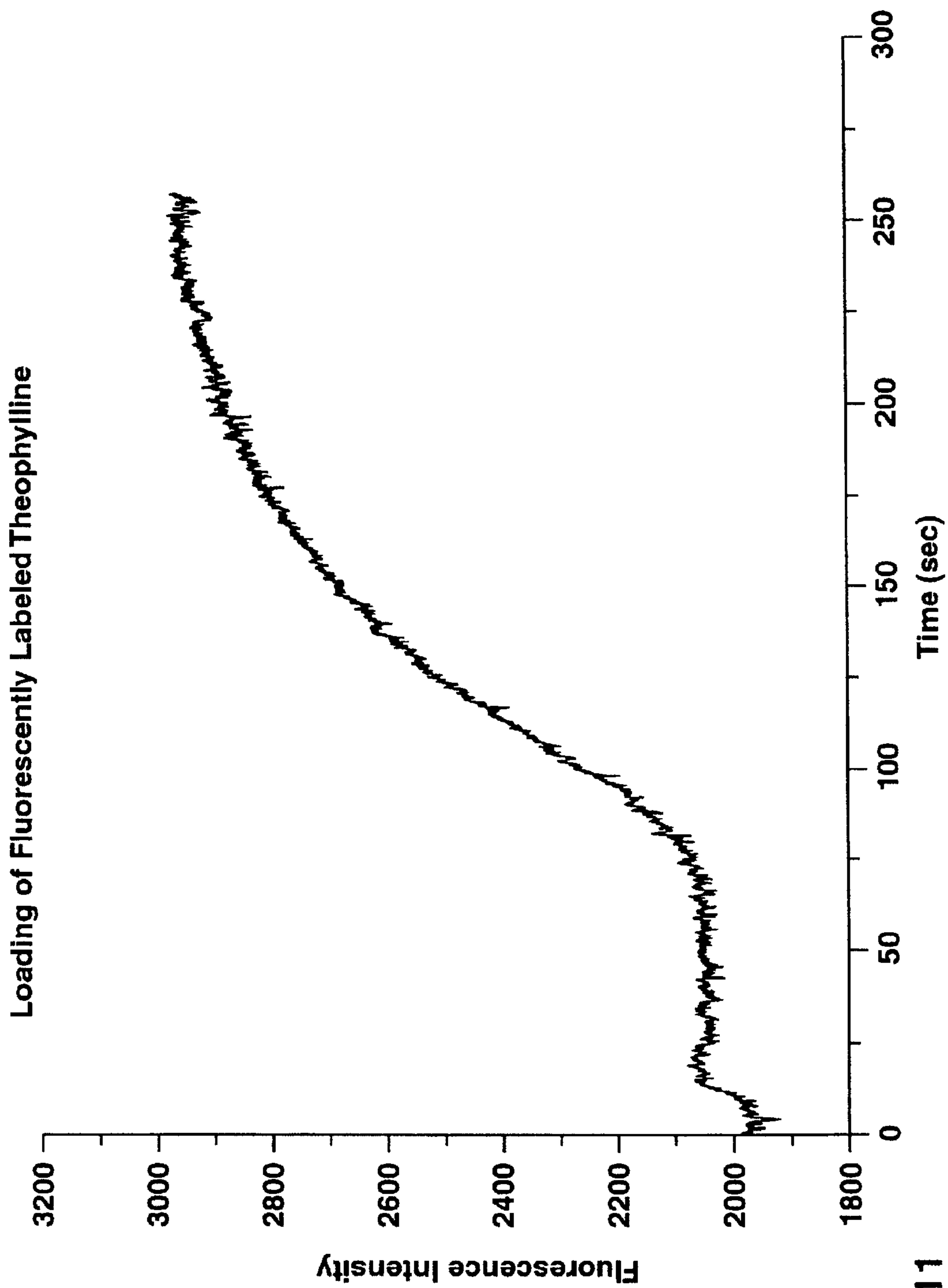


FIG. 11

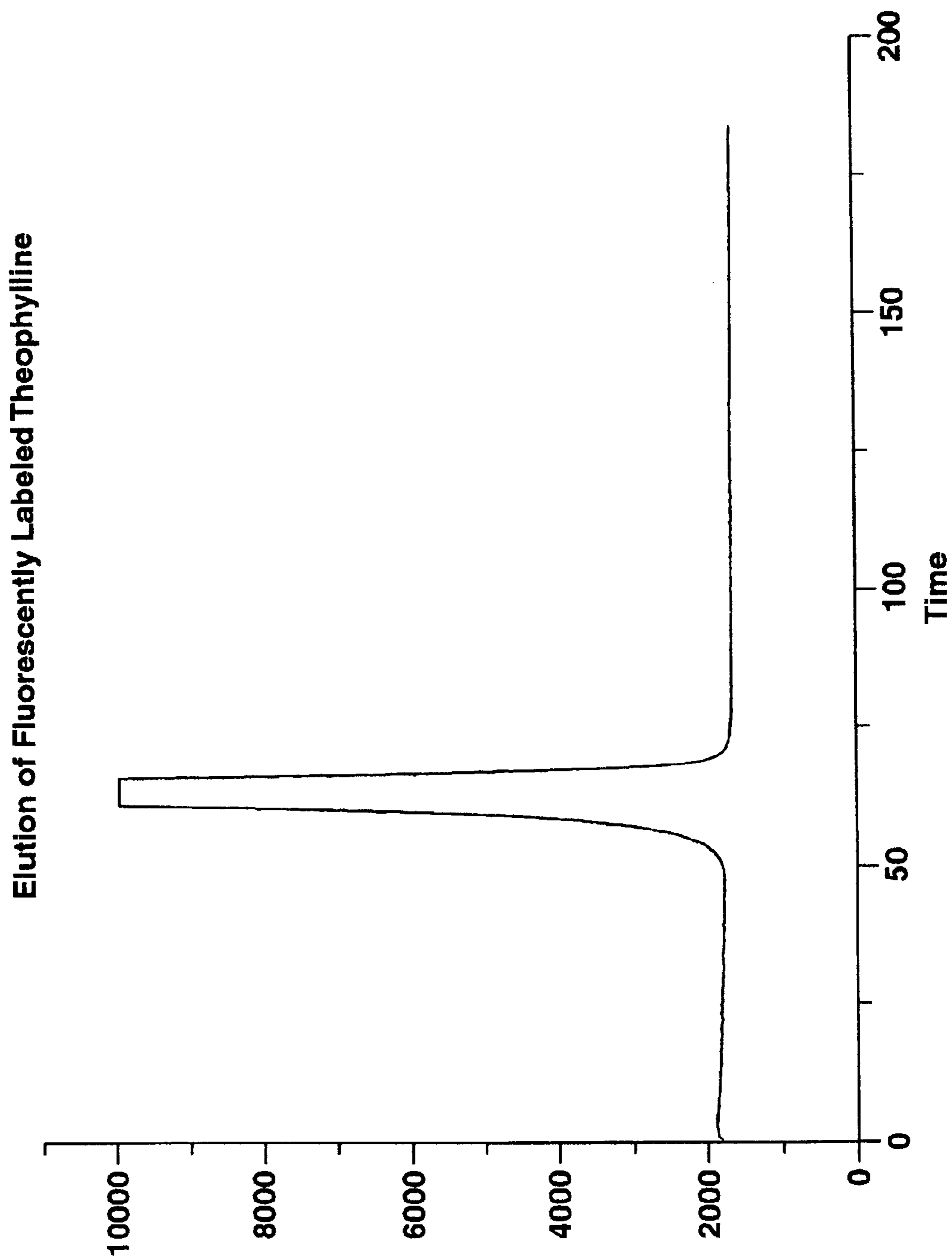


FIG. 12

Decrease in CL With Successive Trials
Using Immobilized HRP

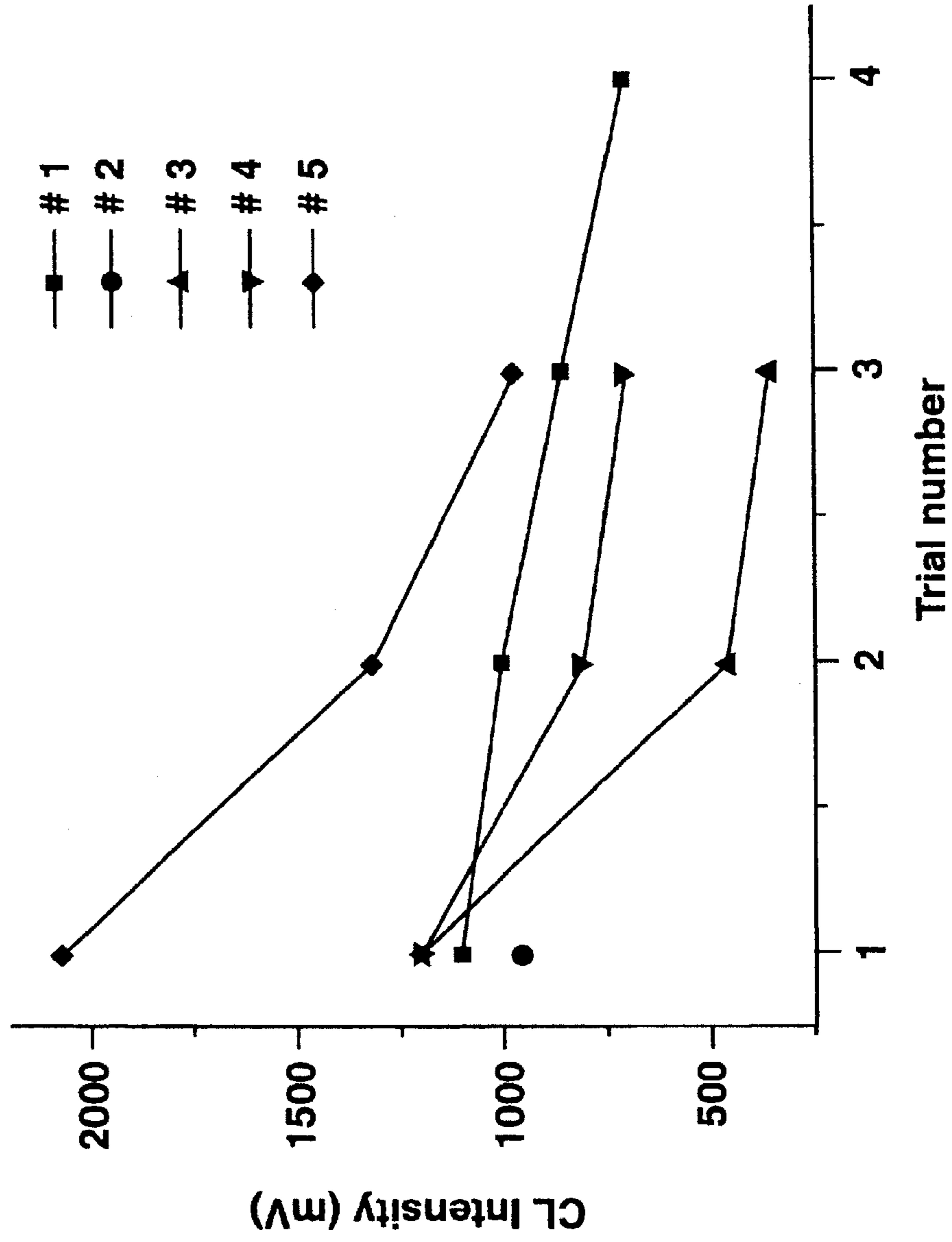


FIG. 13

**APPARATUS AND METHOD FOR TRAPPING
BEAD BASED REAGENTS WITHIN
MICROFLUIDIC ANALYSIS SYSTEMS**

Matter enclosed in heavy brackets [] appears in the original patent but forms no part of this reissue specification; matter printed in italics indicates the additions made by reissue.

FIELD OF THE INVENTION

The present invention relates generally to microfluidic analysis systems, and more specifically to micro-Total Analysis Systems (μ -TAS), for performing liquid phase analysis at a miniaturized level.

BACKGROUND OF THE INVENTION

Recent developments in the field of micro-Total Analysis Systems (μ -TAS) have led to systems that perform chemical reactions, separation and detection at a miniaturized level on a single microchip [see, for example Harrison, D. J. ; Fluri, K.; Fan, Z.; Effenhauser, C.S.; and Manz, A., *Science* 1993, 261, 895-897. Harrison, D. J.; and van den Berg, E.; Eds., *Micro Total Analysis Systems '98, Proceedings of the μ TAS '98 Workshop* (Kluwer: Dordrecht, 1998), Coyer, C. L.; Tang, T.; Chiem, N.; and Harrison, D. J., *Electrophoresis* 1997, 18 1733-1741].

Most prior art microfluidic devices are based on conventional open tubular flow designs and solution phase reagents. While the functionality of these devices has continued to increase, one key feature that is presently lacking in these prior art devices is the ability to effectively incorporate on-chip packed reactor beds, for introduction of packing materials with immobilized reagents or stationary phases. While a few attempts have been made to employ packed reactor beds in some prior art designs, the difficulty of packing portions of a complex microfluidic manifold with packing material (such as microscopic beads) has so far hindered the effective utilization of these reagent deliver vehicles within microfluidic devices. (The difficulty of packing has been well recognized by practitioners in the field [see, for example, Ericson, C; Holm, J.; Ericson, T.; and Hjertén, S., *Analytical Chemistry*.)

In one prior art example, a packed bed chromatographic device with a bead trapping frit was fabricated in a silicon substrate [Oevirk, G., Verpoorte, E., Manz, A., Grasserbauer, M., and Widmer, H. M. *Analytical Methods and Instrumentation* 1995, 2, 74-82]. However, the packing material in this prior art design could not be readily packed or exchanged, thus limiting its utility.

Several authors have also described the difficulties associated with reproducibly fabricating frits for retaining packing material in conventional capillaries [Boughtflower, R. J.; Underwood, T.; Patterson, C. J. *Chromatographia* 1995, 40, 329-335. Van den Bosch, S. E.; Heernstra, S.; Kraak, J. C.; Poppe, H. J. *Chromatogr. A* 1996, 755, 165-177. Colon, L. A.; Reynolds, K. J.; Alicea-Maldonado, K.; Fermier, A. M. *Electrophoresis* 1997, 18, 2162-2174. Majors, R. E. *LC CC* 1998, 16, 96-110.]. The frits used in conventional systems are prepared using time and labor intensive procedures, the most commonly used method involving the use of pure silica gel, wetted down with aqueous sodium silicate. The frit is made by first tapping a capillary end into a paste made from silica and aqueous sodium silicate. The resulting plug of silica is then heated to make a frit.

Furthermore, using frits produced by prior art methods of construction often leads to the formation of undesirable bubbles. Altria, K. D.; Smith, N. W.; and Turnbull, C. H., *Chromatographia*, 46 (1997) 644. Majors, R. E., *LC-GC*, 16 (1998) 96.]Bubbles cause discontinuity within a column, hindering solution flow and ultimately preventing separation from occurring. The bubbles are thought to arise from a change in electroosmotic flow (EOF) velocity caused by moving from a bead trapping frit into an open capillary. The formation of bubbles, which have been observed to increase at higher voltages, also limits the amount of voltage that can be applied across the capillary, thereby limiting column length, separation efficiency, and speed of analysis.

Developing a functional on-chip packed reactor bed design which overcomes the limitations in the prior art would significantly enhance the range of the microfluidic toolbox and extend the number of applications of such devices.

SUMMARY OF THE INVENTION

Generally, the present invention provides an on-chip packed reactor bed design using one or more weir structures that allow for an effective exchange of packing materials (beads for example) at a miniaturized level. The present invention extends the function of microfluidic analysis systems to new applications. For example, the packed reactor bed formed according to the present invention allows on-chip solid phase extraction (SPE) and on-chip capillary electrochromatography (CEC), as explained in detail further below. The design can be further extended to include, for example, integrated packed bed immuni- or enzyme reactors.

More specifically, the present invention provides:
A microfluidic analysis system, comprising:

- a) a substantially planar substrate having an upper surface;
- b) at least one main channel formed into said upper surface, said main channel having first and second ends and a defined direction of flow in use;
- c) a cover plate arranged over said planar substrate, said cover plate closing off said channel from above; and
- d) a first weir formed across said main channel and between said first and second ends of said channel, said first weir providing at least one flow gap to allow, in use, at least some fluid to flow past said first weir while trapping packing material having constituent particles that are generally larger than said flow gap.

The microfluidic analysis system may further comprise at least one side channel formed into said planar substrate, said side channel being connected at a first end to said main channel at a location upstream from said first weir, and at a second end to a reservoir, said side channel providing a higher flow resistance than said main channel.

The microfluidic analysis system may further comprise a second weir located upstream from said connected first end of said side channel, said first and second weirs forming a chamber therebetween, said second weir providing at least one flow gap to allow, in use, at least some fluid to flow past said second weir while trapping said packing material within said chamber.

Each side channel connection to said main channel may be provided with a hook structure whereby, in use, packing material is sprayed into said chamber to facilitate even packing.

Said hook structure preferably at least partially obstructs direct line-of-sight entry of packing material from said side channel into said chamber and forms a chamber mouth to one side of said hook structure.

The present invention also provides a method of packing the chamber in the microfluidic analysis system as claimed above, said method comprising, providing a non-conductive substrate and effecting an electrokinetic flow by applying a relatively high voltage at said reservoir, said reservoir containing packing material, and providing relatively low voltages at said first and second ends of said main channel, whereby, packing material flows from said reservoir into said chamber and is trapped by said first and second weirs.

BRIEF DESCRIPTION OF THE DRAWINGS

For a better understanding of the present invention, and by way of example, reference will now be made to the accompanying drawings, which show preferred embodiments of the present invention in which:

FIG. 1A shows a top plan view of a microfluidic device according to the present invention;

FIG. 1B shows an enlarged perspective view of a chamber in which packing materials (such as beads) are trapped;

FIG. 2A shows a cross-sectional view of the chamber shown in FIG. 1B taken along line A-A, and further shows packing material (beads) which are parked into the chamber and which are retained by a cover plate;

FIGS. 2B and 2C show a side view and end view, respectively, of an alternative embodiment of a weir according to the present invention;

FIG. 3A shows an initial stage of packing material (beads) being packed into the chamber shown in FIGS. 1B and 2A;

FIG. 3B shows the chamber of FIG. 3A after it has been completely filled with packing material (beads);

FIG. 4A shows an early stage of preconcentration of a 10 nM BODIPY solution at the weir/bed interface near the top of FIG. 4A;

FIG. 4B shows a later stage of preconcentration of a 1.0 nM BODIPY solution at the weir/bed interface near the top of FIG. 4B;

FIG. 5 shows a plot of fluorescence intensity vs. time, showing fluorescence of a first 1.0 nM BODIPY sample during loading, followed by a buffer flush, and then preconcentrated BODIPY during elution with acetonitrile (ACN);

FIG. 6 shows an electrochromatogram of BODIPY and fluorescein, showing different steps of the separation including load, flush, and elution;

FIGS. 7A-7D show electrochromatograms of BODIPY and fluorescein with different concentrations of acetonitrile in the mobile phase, specifically at: (a) 30%; (b) 22%; (c) 15%; and (d) 10%;

FIG. 8A-8C show top plan views of alternative embodiments of a microfluidic device according to the present invention;

FIG. 9 shows a top plan view of a microfluidic device according to the present invention having multiple packed chambers;

FIG. 10 shows a schematic view of a microfluidic device according to the present invention being used in conjunction with a mass spectrometer;

FIG. 11 shows a graph plotting the fluorescence intensity of theophylline against time, as it saturates a packed bed;

FIG. 12 shows theophylline being eluted from packed bed in a relatively narrow band; and

FIG. 13 shows each successive trial resulting in lower light generated from the CL reaction.

DETAILED DESCRIPTION OF THE INVENTION

As explained above, the present invention is designed to provide a convenient system and method of trapping packing

materials (such as beads) on-chip, and of effectively packing and unpacking the trapping zones, to provide a functional on-chip packed reactor bed which significantly extends the number of applications of microfluidic analysis devices.

One such extended application facilitated by the present invention is on-chip sample preconcentration by solid phase extraction (SPE). In microfluidic analysis, SPE is often required to overcome detection limit problems, or to eliminate a potential interferent. To date, preconcentration within microchips has been performed by sample stacking using "isoelectric focusing" [Jacobson, S. C. and Ramsey, M. *Electrophoresis* 1995, 16, 481-486]. Advantageously, unlike sample stacking, SPE can be made selective for a particular analyte and does not require precise control of buffer concentrations. For SPE the amount of preconcentration is limited by the preconcentration time, which makes it more flexible than sample stacking. The SPE of an analyte can be beneficial not only for analyte preconcentration, but also for removing other impurities or changing solvent conditions. While the coupling of SPE with microfluidic devices has been accomplished [Figeys, D. and Aebbersold, R. *Anal. Chem.* 1998, 70, 3721-3727], the SPE component in these prior art devices have been made in a capillary or similar cartridge external to the chip, thus resulting in a more complex and more expensive system. The present invention is designed to overcome this prior art limitation by facilitating an on-chip SPE component.

As realized by the present inventors, an integrated, on-chip SPE component is ultimately easier to manufacture, does not require low dead volume coupling to the chip, and eliminates sample handling losses or contamination problems arising from the off-chip sample manipulation required in the prior art. It is anticipated that routine incorporation of SPE onto a chip, as facilitated by the present invention, will reduce problems with on-chip detection limits and will improve the range of sample preparation steps which can be integrated.

Another extended application facilitated by the present invention is on-chip capillary electrochromatography (CEC). CEC has recently received significant attention due to the fact that it combines the separation power of both liquid chromatography and capillary electrophoresis. To date the difficulty associated with packing chromatographic material within devices has focused most previous chromatographic efforts upon prior art open channel methods [Manz, A., Miyahara, U., Miura, J., Watanabe, Y., Miyagi and H. Sato, K. *Sens Actuators* 1990, B1, 249-255; Jacobson, S. C., Hergenroder, R., Koutny, L. B. and Ramsey, J. M. *Anal. Chem.* 1994, 66, 2369-2373; Kutter, J. P., Jacobson, S. C., Matsubara, N. and Ramsey, J. M. *Anal. Chem.* 1998, 70, 3291-3297; He, B., Tait, N. and Regnier, F. *Anal. Chem.* 1998, 70, 3790-3797]. In the prior art, open channel method devices with channel widths of 2 μm or less were required to improve mobile-phase transfer in open columns leading to other practical considerations such as clogging and a short path length for detection. There were also problems with the reproducibility and the cost of stationary phase coating in such structures.

As realized by the inventors, on-chip packed bed chromatography according to the present invention has the benefit of providing low mobile-phase mass transfer, and makes available a wide variety of stationary phases. In this case, the use of an off-chip prepared stationary phase offers the advantage that it eliminates the need for coating the chip and allows for optimization of the stationary phase preparation.

Yet another extended application facilitated by the present invention is providing on-chip bead-based immunoassay and enzyme based assays. These applications are described further below.

To illustrate the present invention by way of example, the inventors conducted a series of experiments, which are described here.

Chip Design

FIGS. 1A and 1B show a microfluidic device **10** as used in these experiments. The device **10** comprises a main channel **11** formed into the top surface of a substrate **8**, and the main channel **11** is separated by a chamber **4**, also formed into the substrate **8**. Two branches of the main channel **11**, as separated by the chamber **4**, are further identified as main reservoirs **1** and **2**. The chamber **4** is connected to a packing material reservoir **3** by a narrow side channel **5**. The packing material reservoir and the narrow side channel **5** are also formed into the substrate **8**. FIG. 1B shows an enlarged image of the chamber **4** obtained with a scanning electron microscope (Jeol X-Vision JSM6301FXV, Peabody, Mass.). the chamber **4** is formed by providing two weirs **6**, **7** formed across the main channel **11** at a relatively narrow portion of the main channel **11** (FIG. 1A). As can be seen from FIG. 1B, the weirs **6**, **7** are not as high as the main channel **11** is deep, so that some fluid is allowed to flow over the weirs **6**, **7**, as explained below. The device **10** was prepared in Corning 0211 glass by the Alberta Microelectronic Corporation (Edmonton, AB), using known chemical etching procedures [Fan, Z. H.; Harrison, D. J. *Anul. Chem.* 1994, 66, 177-184]. It is noted that this substrate material is non-conductive, but if other than electrokinetic forces are being used (as detailed further below), then the substrate material may be semiconducting or conducting. Two photomasks were required to create device **10**: a first photomask was used to etch the tops of the weirs **6**, **7** to a depth of approximately 1 μm ; and a second photomask was used to etch the channels **5**, **11** to a depth of approximately 10 μm .

FIG. 2A shows a cross-sectional view of the weirs **6**, **7** which are not as high as the channel **11** (main reservoirs **1**, **2**) is deep, and thus small flow gaps **14**, **15** are provided between the top of the weirs **6**, **7** and a cover plate **9** (not shown in FIG. 1A or 1B) which is placed on top of the substrate **8**, thereby closing off the chamber **4**, channels **5**, **11** and reservoirs **1**, **2**, **3**. As can be seen in FIG. 2A, the beads **12** are generally larger than the flow gaps **14**, **15** and therefore cannot escape from the chamber **4**.

FIGS. 2B and 2C show a side view and an end view, respectively, of an alternative embodiment of a weir **6'** in which substantially vertical notches **6''** are provided so that the weir **6'** provides less flow impedance. The vertical notches **6''** should be narrow enough that no beads can pass through them (i.e. they should be at least about 10% smaller than the smallest bead diameter).

Solutions and Reagents

Various solutions and reagents were used in these experiments. Acetonitrile (BDH, Toronto, ON) was filtered through a 0.45 μm Nylon-6,6 filter (Altech, Deerfield, Ill.) prior to use. Otherwise, the acetonitrile was used as received, with no added electrolyte. Also, 50 mM potassium phosphate (pH 7.0) and ammonium acetate (pH 8.5) buffers were prepared in ultra-pure water (Millipore Canada, Mississauga, ON). A 1:1 (v/v) mixture of acetonitrile and buffer was prepared. A stock solution of 0.10 mM, 4,4-difluoro-1,3,5,6,7,8 penta methyl-4-bora-3a4a-diaza-s-indacene, BODIPY 493/503 (Molecular Probes, Eugene, Ore.) was prepared in HPLC grade methanol (Fisher, Fair Lawn, N.J.) A 1 mM stock solution of fluorescein di-sodium salt (Sigma) was prepared in phosphate buffer. Both stock solutions were then diluted in the 50 mM phosphate and 50 mM ammonium acetate buffers to give 1.0

μM solutions, which were then diluted to 1.0 nM. This 1.0 nM solution served as the sample for preconcentration and electrochromatography. All aqueous (buffer and sample) solutions were filtered through a cellulose acetate syringe filter (0.2 μm pore size) (Nalgene, Rochester, N.Y.) prior to use.

Packing Material

One suitable packing material used in these experiments comprises a revers-phase chromatographic stationary resin. The resin was Spherisorb ODS1 (Phase Separations, Flintshire, UK), a porous C-18 resin whose particles ranged from 1.5 to 4.0 μm in diameter, as determined by scanning electron microscopy (ODS beads **12**). A slurry of approximately 0.003 g/mL of ODS1 was prepared in acetonitrile. This slurry was used to supply the packing material reservoir **3**, to subsequently pack the chamber **4**.

Certain solvent and additive combinations were found to help the packing material stay in the packed chambers. For example, if ODS beads are introduced in acetonitrile they flow readily, while subsequently switching to an aqueous or predominantly aqueous solvent causes the beads to aggregate and become trapped within the chamber. With ODS beads up to 30% acetonitrile could be present in the aqueous solution without disrupting the aggregation observed to the point of de-stabilizing the packed bed. Up to 50% acetonitrile could be present with only modest loss in aggregation and weak demobilization of the bed. As another example protein G or protein A coated beads formed aggregates in aqueous solution, which made it hard to introduce them into the trapping zone. However, the addition of a neutral surfactant such as Tween 20 or Brij 35 (both are trademarks) prevented such aggregation and allowed the beads to be introduced. Conversely, subsequent removal of the surfactants from the aqueous solvent resulted in aggregation and enhanced stability of the trapped bed. The following trend was observed: when using non-polar or partially non-polar bead phases (for example, ODS and protein coated beads) lowering the surface tension of the solvent from that of water or buffered water, by the addition of organic additives such as organic solvents or surfactants, reduced the tendency to aggregate. Conversely reducing or eliminating materials with lower surface tension from aqueous solution increased the tendency to lock the beads in place on the bed, creating a "solvent lock" method to enhance bead trapping within these devices. Other organic solvents other than acetonitrile, miscible with water may also be used for these purposes, such as methanol, ethanol, dimethylsulfoxide, propylene carbonate, etc. Charged surfactants may also be used instead of neutral surfactants, so long as they are compatible with the proteins that may be present on the beads or in the sample.

Magnetic beads used for magnetic packing may comprise AbeBaw—protein "A" coated beads: composition 36-40% magnetite dispersed within a copolymer matrix consisting of styrene and divinyl benzene (Prozyme, Calif.) Also, Guifeng-oligo (dT)₂₅ coated beads may be used for the isolation of mRNA. The beads have an even dispersion of magnetic material (Fe₂O₃ and Fe₃O₄) through out the bead. The beads are coated with a polystyrene which encases the magnetic material (Dynal, Oslo, Norway).

Instrumentation

Various instruments were used in conducting the present experiments. As these instruments and their operation are well known to those skilled in the art, only a brief description is provided, and the instruments are not shown in the figures.

A power supply and relay system used to control the electrophoretic voltages necessary for bead packing and all liquid handling on-chip has been described previously [Fluir, K., Fitzpatrick, G., Chiem, N. and Harrison, D. J. *Anal. Chem.*

1996, 68, 4285-4290]. LabVIEW programs (National Instruments, Austin, Tex.), were written for computer control of the voltage system and for data acquisition.

A laser-induced fluorescence detection system used in this experiment consisted of a 488 nm argon ion laser (Uniphase, San Jose, Calif.), operated at 4.0 mW, and associated focusing optics [Manz, A., Miyahara, Y., Miura, J., Watanabe, Y., Miyagi, H. and Sato, K. *Sens. Actuators* 1990, B1, 249-255] (Melles Griot, Irvine, Calif.). Fluorescence emitted from the BODIPY sample (as described above) was collected by a 25 X, 0.35 NA microscope objective (Leitz Wetzlar, Germany). The images were observed with a SONY CCD-IRIS camera. Alternatively a 530 nm emission filter and a photo multiplier tube (PMT) (R1477, Hamamatus, Bridgewater, N.J.) were used as a detector positioned so that the narrow channel **5** between the chamber **4** and packing material reservoir **3** could be monitored. Data were collected from the section of main channel **11** just next to the chamber **4**. The weir **6** was just out of the field of view. The PMT was biased at 530 V while the PMT signal was amplified, filtered (25 Hz Butterworth) and sampled at a frequency of 50 Hz.

The fluorescence of the buffer, acetonitrile, and 1.0 nM BODIPY in both buffer and acetonitrile was measured using a Shimadzu RF-5301PC Spectrofluorophotometer.

While specific models and manufacturers have been provided for various instrumentation described above, it will be understood by those skilled in the art that any suitable, functional equivalent may be used.

Chip Operation

Referring back to FIGS. 1A and 1B, the narrow side channel **5** leading into the chamber **4** from packing material reservoir **3** was used to direct stationary phase packing material into the chamber **4** using electrokinetic pumping [Yan, C., U.S. Pat. No. 5,453,163, 1995; Knox, J. J. and Grant, I. H. *Chromatographia* 1991, 32, 317-328]. As mentioned above, the substrate **8** is non-conductive, which allows packing of the beads **12** using the electrokinetic pumping method.

The device **10** was not conditioned with any aqueous solutions prior to use. The chamber **4**, channels **5**, **11**, and reservoirs **1**, **2**, **3** were first filled with acetonitrile. The chamber **4** was packed with ODS beads **12** (FIG. 2) by replacing the acetonitrile in packing material reservoir **3** with the ODS/acetonitrile slurry (described above), then applying positive high voltage at packing material reservoir **3** while holding main reservoirs **1** and **2** at ground. The voltage applied at packing material reservoir **3** was sampled from 200 V to 800 V over approximately 5 min to effect packing of chamber **4**.

Once the chamber **4** was packed, a step gradient was performed to introduce aqueous solution to the main channel **11** and the ODS beads **12** in the chamber **4**. A 1:1 (v/v) mixture of acetonitrile and buffer was placed in reservoirs **1** and **2**. Acetonitrile replaced the slurry in packing material reservoir **3**. A voltage was then applied to main reservoir **1** and was ramped from 200 V to 800 V, with packing material reservoir **3** biased at 400 V and main reservoir **2** grounded. After 2 to 5 min at 800 V, the acetonitrile/buffer mixture in reservoirs **1** and **2** was replaced with buffer, and the same voltage program repeated. The chamber **4** was monitored visually to ensure that the acetonitrile was completely replaced by buffer and that the packing material (beads **12**) did not shift or unpack during this procedure. (The beads **12** could be seen to agglomerate as the acetonitrile was expelled, and the index of refraction change at the water/acetonitrile interface was clearly visible.) The experiments conducted are described in further detail below.

Experimental Results and Discussion

In order to conduct the experiments, it was necessary to pack the chamber **4** with packing material (beads **12**), as shown in FIG. 2A.

The narrow side channel **5** shown in FIGS. 1A and 1B was made to be about 30 μm wide to supply packing material (beads **12**) to the chamber **4**. A sample could then be delivered from reservoir **2** (the inlet channel), across the chamber **4** and on towards main reservoir **1** (the outlet channel). The volume of the chamber **4** was 330 pL, while the volume of the outlet and inlet channels was 1.5×10^{-8} L, respectively. The main channel **11** had much lower flow resistance than the side channel **5**, in spite of the weirs **6**, **7**, given their relatively wide widths (580 μm , tapering to 300 μm at the weirs) in comparison to the width of the narrow channel **5** (30 μm). The relative flow resistance in the device **10** was manipulated by the selection of the width dimensions for these channels **5**, **11** in order to encourage flow between main reservoirs **1** and **2**, rather than into the narrow bead introduction side channel **5** during sample loading and elution.

Reverse phase ODS beads **12** (as described previously) were used in the SPE device because of their extensive use for the chromatography of proteins, peptides and tryptic digests [Seifar, R. M.; Kok, W. T.; Kraak, J. C.; and Poppe, H. *Chromatographia*, 1997, 46, 131-136. Yan, C.; Dadoo, R.; Zhao, H.; Zare, R. N.; and Rakestraw, D. J., *Anal. Chem.* 1995, 67, 2026-2029.] as well as other applications of SPE and CEC [Nielsen, R. G.; Riggan, R. M.; Rickard, E. C. *J. Chromatogr.* 1989, 480, 393-401. Hancock, W. S.; Chloupek, R. C.; Kirkland, J. J.; Snyder, L. R. *J. Chromatogr. A* 1994, 686, 31-43.] Electrokinetic packing of conventional capillaries has been described previously, [Yan, C.; U.S. Pat. No. 5,453,163, 1995. Knox, J. H.; Grant, I. H. *Chromatographia* 1991, 32, 317-328.], and the inventors have adapted the method for the present invention.

As briefly explained earlier, the packing procedure involved applying a positive voltage (ramped from 200-800 V) to the packing material reservoir **3**, while grounding main reservoirs **1** and **2**. The applied voltage induced EOF to flow down the bead channel, carrying the beads into the cavity. An organic solvent was required to suspend the chromatographic beads **12** to prevent them from aggregating and plugging the narrow side channel **5**. Studies have shown that capillaries filled with acetonitrile exhibit substantial electroosmotic flow [Wright, P. B.; Lister, A. S.; Dorsey, J. G. *Anal. Chem.* 1997, 69 3251-3259, Lister, A. S.; Dorsey, J. G.; Burton, D. E. *J. High Resol. Chromatogr.* 1997, 20, 523-528. Schwer, C.; Kenndler, E. *Anal. Chem.* 1991, 63, 1801-1807. Salimi-Moosavi, H.; Cassidy, R. M. *Anal. Chem.* 1995, 67, 1067-1073.]

As shown in FIG. 3A, at the early stages of packing, the beads **12** entering the chamber **4** contacted the weirs **6**, **7** on either side of the chamber **4**. As explained earlier, the beads **12** are unable to traverse the weirs **6**, **7** because the distance from the top of the weirs **6**, **7** to the bottom of the cover plate **9** (approximately 1.0 μm) is less than the diameter of the individual particles of the ODS beads **12** (approximately 1.5-4.0 μm).

As shown in FIG. 3B, the chamber **4** continued to pack until it was entirely filled with chromatographic material. As discussed earlier, the difficulties associated with reproducibly fabricating frits for retaining packing material is well known. Importantly, the weir design used in the present invention circumvented this problem, and the electrokinetic packing of the beads provided an even distribution of beads throughout

the chamber with no observable voids. In fact, the use of weir structures may ultimately eliminate the need for on column frit fabrication.

The weir design of the present invention allows electric fields to be applied across the trapping zone formed by two weirs, when filled with beads, in a range as high as 20,000 to 80,000 V/cm without bubble formation at the weir. Separations performed in devices with these weirs can use electric fields at least as high as 15,000 V/cm. The power dissipated across a weir can be as high as 3-7 W/m without the formation of bubbles. In contrast frits formed in conventional columns have at the best been reported to form bubbles at power dissipations above 0.6 W/m, and electric fields in the range of 150-600 V/cm are the best that have been reported without bubble formation.

It is possible to couple an external capillary to a chip and allow the weir to be used as the trapping element for the beads packed within the external electrochromatography capillary. This can be accomplished using a low dead volume coupling, such as described by Bings et al. (N. H. Bings, C. Wang, C. D. Skinner, C. L. Colyer, P. Thibeault, D. J. Harrison, *Anal. Chem.* 71 (1999) 3292-3296.) In this way the chip based weir can replace the frits normally formed within external capillaries, and allow higher electric fields to be used, improving speed and separation efficiency.

(It is noted here that it was also possible to pack the cavity by applying a vacuum at main reservoirs 1 and 2, although this was less convenient when electrokinetic flow was used for sample loading and elution).

If for some reason the beads 12 did not pack as tightly as was desirable (as shown in FIGS. 3A and 3B) they were removed from the chamber 4 by simply reversing the voltages, and the packing procedure was then repeated. It is noted that once an aqueous solution was introduced to the chamber 4, the reverse-phase beads 12 tended to aggregate and were more difficult to remove. However, subsequent removal was accomplished by flushing the aqueous solution out with acetonitrile, using either EOF or vacuum, or a combination of the two. Advantageously, the ability to effectively remove the beads 12 from chamber 4 allowed used chromatographic beads to be refreshed, or a more applicable material to be substituted.

Significantly, a design utilizing a hook structure 13 at the chamber entrance (FIGS. 1B and 3A) yielded the most favorable results in packing, enabling the chamber 4 to be packed and remain so after removal or alteration of voltages or vacuum. As seen from the figures the side channel 5 connects to the chamber 4 via a chamber mount 4A in an asymmetric fashion, relative to the weirs 6, 7. Also, the hook structure 13 preferably obstructs direct line-of-sight entry of packing material from the side channel 5 into the chamber 4. Rather, the hook structure 13 forces packing material to enter the chamber 4 indirectly via the chamber mouth 4A.

As explained earlier, during the packing step, the packing material reservoir 3 has a positive bias applied with reservoirs 1 and 2 grounded. The inventors believe that the hooked structure 13 causes electric field lines to follow a curved pathway into the cavity. Consequently, as the chromatographic beads 12 follow the electric field lines into the chamber mouth 4A they appear to be "sprayed" as if from a snow blower (FIG. 3A), to become uniformly packed.

During the packing procedure the chamber 4 filled only to the beginning of the hook structure 13 (see FIG. 3B). Once filled, the beads were observed to flow down the sides and up the middle of the narrow side channel 5 (toward packing material reservoir 3) mimicking the solvent back flow generated in a closed electrophoretic system [Shaw, D. J. Introduc-

tion to Colloid and Surface Chemistry, 3rd ed. Butterworths: London, 1980.]. In such a closed system, EOF is directed along the walls until it reaches the end of the chamber, where pressure causes the solution to reverse direction and flow back up the center of the bead introduction channel.

A key aspect of the hooked structure as shown is the asymmetric entrance into the trapping zone, which allows for better packing. A symmetric entrance means the entering beads can go to both weir equally, which tends to lead to uneven or difficult packing. An asymmetric structure allows the beads to pack preferentially at one end of the trapping zone first and then build up in one direction from that location. The key role of the hook structure is to prevent line-of sight outflow from the trapping zone during use of the packed bed.

Chambers constructed without an asymmetry in the entrance were not observed to pack as well as asymmetric entry designs. In these cases, packing material tended to fill the corners furthest from the entrance, but no additional material would enter the chamber. The inventors believe that, due to its symmetric design, this type of chamber exhibits solvent back flow, after it has filled to a certain extent. That is, the partially filled chamber may resemble a closed or restricted system. Such an occurrence would preclude the filling of the symmetric chamber with beads and is consistent with previously observed behavior, as explained by Shaw. Such behavior may account for the ability to fill symmetric structures on some occasions but less readily on others. In contrast, an asymmetric design, with or without a hook structure 13 guarding the entrance is less likely to experience back flow directly into the narrow bead introduction channel 5.

Solid Phase Extraction (SPE) On-Chip

As explained earlier, the present invention allows applications of microfluidic analysis systems to be extended. One such extension is facilitating SPE directly on-chip. Preconcentration is a valuable tool that can be used to enhance the sensitivity of microfluidic devices. To determine the ability of a packed SPE bed constructed on a microchip to preconcentrate an analyte, we concentrated a 1.0 nM solution of BODIPY reagent from 50 mM phosphate buffer. Solution conditions utilized were similar to those used for protein and peptide analysis in HPLC-CE systems. [Bushey, M. M.; Jorgenson, J. W. *Anal. Chem.* 1990, 62, 978-984. Castagnola, M.; Cassiano, L.; Rabino, R.; Rossetti, D. V. *J. Chromatogr.* 1991, 572, 51-58.] The BODIPY reagent, when diluted in aqueous buffer, exhibits a high affinity for ODS material and is an excellent fluorophore. The preconcentration and elution of the BODIPY reagent was carried out in four steps: equalization of the SPE bed with buffer; sample introduction; buffer flush; and elution of analyte.

Following rinsing of the packed bed with phosphate buffer, a solution of 1.0 nM BODIPY was placed in main reservoir 1, and +200 V was applied for 2 minutes, with main reservoir 2 grounded. The EOF (0.2 mm/sec, 1.2×10^{-9} L/sec) flowed towards reservoir 2, carrying the BODIPY onto the SPE bed during the loading step.

As shown in FIG. 4A, fluorescence of the adsorbed BODIPY occurred initially at the first few layers of beads 12 only (near the top of the Figure). FIG. 4B shows the SPE bed after 1.5 minutes, with a total of 1.4×10^{-16} moles of BODIPY reagent loaded on the bed (assuming complete capture of the dye). No sample breakthrough was observed with BODIPY, due to its high affinity for the ODS material. In fact, visual observation indicated that after concentrating 1.0 nM BODIPY solution for two minutes only 5% of the physical volume of the SPE bed was utilized suggesting that the capacity of the 330 pL bed was about 2.8×10^{-15} moles of analyte.

A buffer wash step was used after loading to wash sample remaining within the channel **11** onto the bed (in chamber **4**). The solutions in reservoirs **1** and **2** were then replaced with acetonitrile, and the dye was eluted with solvent moving in the same direction as the initial loading step (or by reversal of the potential gradient during the elution step, it could be directed back towards the original sample reservoir). Both procedures work well, but the latter was more convenient for our testing. FIG. **5** shows graphically the 3-step preconcentration experiment for a 1.0 nM BODIPY sample following bed equalization. The 90 second loading step showed an increase in signal as the fluorescent sample passed by the detector positioned as shown in FIG. **1A**. This was followed by a 60-second rinse step. Acetonitrile was then used to elute the BODIPY reagent off the bed in the opposite direction to which it was loaded, eliminating the need for detector repositioning. The BODIPY reagent eluted in a relatively narrow 3-second band following a 90-second preconcentration step exhibiting a many fold concentration increase compared to the original sample. The fluorescence of the BODIPY (1.0 nM) reagent was tested in both buffer and acetonitrile and did not show a significant difference in intensity for either of the solvents. The preconcentration factor (P.F.) can be estimated using equation (1):

$$\text{P.F.} = \frac{V_i}{V_f} = \frac{t_{pre} \cdot f_{buff}}{t_{elute} \cdot f_{elute}} \quad (1)$$

where V_i is the volume of buffer containing analyte and V_f is the volume of acetonitrile containing analyte. The volume V_i is the product of the preconcentration time (t_{pre} , sec.) and the electroosmotic flow of the sample being concentrated (f_{buff} , L/sec.) while V_f is the product of width of the eluted analyte peak (t_{elute} , sec.) and the flow rate of the eluting solvent f_{elute} (L/sec). For this case, the analyte was preconcentrated by a factor of at least 100 times. After sufficient concentration the BODIPY is easily observed visually on the SPE bed.

Different sample loading times were utilized to increase the amount of preconcentration. In the experiments, reconcentration times ranging from 120-532 seconds were studied yielding preconcentration factors of 80-500. Peak area (rsd 3-11%) plotted versus preconcentration time yielded a linear relationship ($r^2=0.9993$) over the studied conditions.

Capillary Electrochromatography (CEC) On-Chip

As explained earlier, another application facilitated by the present invention is on-chip capillary electrochromatography (CEC). Reversed phase mode CEC was performed on a chamber **4** packed with octadecyl silane beads **12** equilibrated with buffer. Due to the lack of an injector within the chip design, the samples were loaded onto the front of the chromatographic bed in 50 mM ammonium acetate buffer, pH 8.5 (see "Solutions and Reagents," above). Both compounds were totally retained under these conditions, as indicated by a lack of analyte signal in the loading and flush steps. The loading step functioned to both introduce the sample and preconcentrate the retained analytes at the front of the bed [Swartz, M. E.; Merion, M.; J. Chromatogr, 1993, 632, 209-213.] FIG. **6** shows the three steps involved in the CEC separation of BODIPY and fluorescein with a mobile phase composition of 30% acetonitrile/ 70% aqueous 50 mM ammonium acetate. Once the mixed mobile phase reaches the bed, both compounds begin to undergo chromatography and are eluted from the bed. The compounds are completely eluted and separated in less than 20 sec on less than 200 μm of chromatographic bed, yielding a plate height of 2 μm ($N=100$ plates or 500 000

plates/m) for the fluorescein peak. Under these conditions, the fluorescein is eluted prior to the BODIPY reagent. Peaks were identified by comparing retention times of the standards with those of the mixture. At pH 8.5 fluorescein possesses a net (-2) charge while BODIPY is neutral. In a normal CZE separation the electrophoretic mobility of fluorescein would oppose the EOF, causing the BODIPY to elute prior to fluorescein. In this case the elution order of the two components is reversed, indicating an interaction between the analytes and the stationary phase. The BODIPY being more hydrophobic has a higher affinity for the chromatographic material than does fluorescein causing the BODIPY to be retained more and eluted later.

Finally, FIGS. **7A-7D** shows the CEC separation of BODIPY and fluorescein utilizing mobile phases with different concentrations of acetonitrile. It was observed that the increased acetonitrile concentration lowers the polarity of the mobile phase, decreasing the amount of time required for the BODIPY to elute. The elution time for fluorescein does not change, indicating little to no chromatographic retention except at low % acetonitrile. Decreasing the acetonitrile concentration provides baseline resolution, but leads to more extensive band broadening.

Our present results are comparable to that reported for open tubular CEC on a chip [Jacobson, S. C., Hergenröder, R., Koutny, L. B., Ramsey, J. M. Anal. Chem. 1994, 66, 2369-2373. Kutter, J. P.; Jacobson, S. C.; Matsubara, N.; Ramsey, J. M. Anal. Chem. 1998, 70, 3291-3297. Ho, B., Tait, N., Reginier, F. Anal. Chem. 1998, 70, 3790-3797.]

Immunoassay Using Bead-based Reagents

Immunoassay on beads, or immunosorbent assays involves placing either an antibody or antigen on the surface of the bead. As a solution containing an antigen passes over the beads, the antigen specifically binds the antibody. In this way the specificity of the antigen for the antibody is utilized to separate it from other species in solution. Later the solution conditions are changed so that the antibody or antigen is eluted from the beads and is detected as either complex or the free antibody. The development of immunosorbent assays on chip is attractive because of the small amounts of reagents that are consumed. In addition microchips offer very fast analysis times compared to conventional methods performed in micro titer plates or in syringes packed with immunobeads. Immunosorbent assays on-chip also provide lower concentration detection limits than solution phase immunoassays on-chip. Making the development of bead based immunoassay on-chip important.

Beads that have specific enzymes linked to them are packed into the chamber created by the two weirs. The use of beads is preferential because of the increased surface area of the beads as opposed surface area of the channel walls. The higher surface area leads to a greater capacity and more efficient trapping of the analyte. The weirs form a well-defined chamber for the immunoassay beads to pack. We have demonstrated bead-based immunoassay on chip for the enzyme theophylline. In the experiment magnetic beads coated with protein A are packed within the chamber of the chip. Later the antibody (antitheophylline) is flowed across the bed in a 1 mM tricine buffer pH 8.0. When the antitheophylline flows through the packed bed the antibody binds to the protein A. The antitheophylline was passed over the bed for several minutes to ensure that the bed is saturated with antibody. A buffer washing step was then utilized to remove the remaining unbound antibody from the chamber and channels. The bed was then saturated with fluorescently labeled theophylline (diluted from a kit) by flowing it through the bed where it binds to the antitheophylline. The point at which the

bed was saturated was determined by monitoring fluorescence below the bed and determining the point where the breakthrough curve plateaus. Following breakthrough the theophylline solution is washed from the device using a buffer flush step. A chaotropic agent is then added to elute the theophylline from the bed as either free protein or theophylline/antibody complex. Chaotropic agents can be of various types, however in this example a mixture of 90% acetone/10% tricine buffer was used. Once the chaotropic agent reaches the packed bed the theophylline is eluted in a relatively narrow band.

Although normally under these circumstances a competitive assay would be performed the direct assay demonstrates the ability of the chamber on the weir device to act as an immunoassay bed.

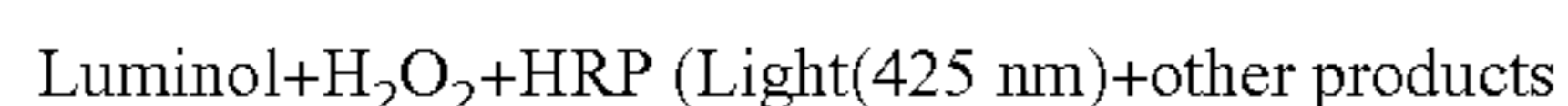
Enzyme Reactor Beds

There have been several methods developed for immobilizing enzymes onto solid supports like beads. Once immobilized the enzyme beads can be packed into beds to perform chemical reactions on solutions as they are flowed through them. Normally a solution containing a substrate is passed through the bed. When the substrate comes in contact with the enzyme reacts with the substrate to yield a product. The product resulting from the reaction of the immobilized enzyme and substrate can be later used as a method of detection or in other synthetic processes. This example illustrates the use of the immobilized enzyme horse radish peroxidase (HRP) and xanthine oxidase(XO) on porous silica beads (5 μ m diameter). These results show that enzymes, once immobilized onto beads, can be trapped/packed into the weir device, where they are still active and can be used as an enzyme reactor bed.

XOD and HRP were immobilized onto Nucleosil 1000-5 silica beads (Machrey-Nagel, Germany) that has been silanized with 3-aminopropyltriethoxysilane, by crosslinking with gluteraldehyde (Sigma). The immobilization of enzymes on glass beads has been described previously and is known by practitioners of the art. All studies were performed using 50 mM boric acid adjusted with 1 M NaOH to pH 9.

The immobilization of HRP and XOD was performed to demonstrate two principals. First was the ability to pack the enzyme immobilized beads within the weir device and then second was to demonstrate that the enzyme was still active and could be utilized to catalyze reactions once packed. To show each of these principals a chemiluminescent reaction was performed using the weir device.

The ability to pack immobilized enzymes allows different methods of detection to be used for certain analytes. For example the luminol chemiluminescence (CL) reaction can be used for very sensitive determinations when only small amounts of analyte are available or when labeling reactions are otherwise difficult to perform. CL reactions are unique in that they do not require a light source simplifying the detection scheme. The chemiluminescence reaction catalyzed by HRP is shown below.



Beads immobilized with HRP were packed into the weir device and a solution containing the reagents for the reaction passed through the bed. The immobilized HRP was found to catalyze the chemiluminescent reaction when a solution of H_2O_2 (100M) and luminol (10 mM) was flowed over a bed that had been packed with beads containing immobilized HRP. Light generated from the reaction was detected downstream from the enzyme bed.

However, it was noticed that with each successive trial the light generated from the CL reaction was lower than in the

previous trial. This is probably caused by a decrease in the activity of the enzyme with each successive run. These results evidence the advantage of a method of removing the exhausted beads and replacing them with fresh ones, such as discussed for the replacement of ODS beads within the weir device.

Alternative Embodiments

While a two weir embodiment of the design according to the present invention has been described above, other embodiments are also possible. For example, it is possible to implement a single weir design to form an on-chip reactor bead (i.e. not having a second weir 6 located upstream in the main channel 11). Specifically, by providing a downstream weir 7 formed across the main channel and providing pressure only in a downstream direction (i.e. from main reservoir 2 and side channel 5 to main reservoir 1), it has been observed that packing can be achieved against the downstream weir 7. However, it is noted that a single weir design may result in the formation of a ragged leading edge for the packed bed that reduces separation efficiency when used for SPE or CEC. Additionally, the high back pressure associated with a long bed of small beads limited the length of the pack to about 4-6 mm. A high pressure fitting for the microchip would allow high pressure pumping and allowing somewhat greater lengths.

Other types of forces may also be used to create a packed bed using a single weir design. For example, it was also possible to achieve a limited degree of packing (to a length of a few millimeters) using electrokinetic forces, directed only in a downstream direction (i.e. from main reservoir 2 and packing material reservoir 3, to main reservoir 1).

In addition to varying the numbers of weirs, it is also possible to provide more than one inlet or outlet to a chamber, as shown in alternative embodiments of the present invention in FIGS. 8A-8C.

In FIG. 8A, a chamber 4 is formed between two weirs 6, 7. Two side channels 5a, 5b are provided to serve as an inlet or outlet to the chamber 4. As shown in FIG. 8A, the side channels 5a, 5b may be offset relative to each other to better facilitate packing of the chamber. A second side channel is added to allow the beads to be flushed out to waste at the other end of the trapping zone, or to allow the flushing agent to be delivered from an alternate reservoir. The latter design can prevent used beads from contaminating the fresh bead stream, and/or prevent sample and sample waste solutions from being directed into the trapping zone during flushing.

As shown in FIG. 8B the side channel in this design may have one or more optional branches 5c, to allow the side channel 5b to be flushed of beads, or to allow beads being flushed out of the trapping zone to be directed, for example, into a waste reservoir instead of into the packing material reservoir 3 (not shown).

Another embodiment is shown in FIG. 8C, in which a side channel weir 16 is provided near the entrance of a third side channel 5d to the chamber 4, to allow fluid flow without passage of beads. This "weired" side channel 5d may be used, for example, to release pressure build up in the chamber 4 during loading of the beads, particularly when the length of the chamber 4 (as measured between the weirs 6, 7) is greater than 4-6 mm.

In all three embodiments shown in FIGS. 8A-8C, the side channel entrance into the chamber 4 may be modified to include a hook or similar shape, as described earlier, in order to prevent direct "line-of-sight" flow from a side channel into the chamber 4, or vice versa. As explained earlier, this entrance modification serves to spray the beads into the trap-

15

ping zone in order to assist packing, and to reduce the tendency of the beads to exit from the chamber 4 during later use.

Loading of beads with more than one side channel, as shown in FIGS. 8A-8C, is performed in a manner similar to that for a single side channel, two weir design, (as described above) except that a potential must also be applied to the additional side channels to prevent flow into those side channels when using electrokinetic loading. During removal of the beads a voltage may be applied to a second side channel (e.g. side channel 5b in FIG. 8A) to drive beads out of the trapping zone or chamber 4, applying voltage potentials such as those used with the single side channel design but adjusted for the potential drop in the additional side channel. As will be appreciated, the direction of flow during the flushing step can be controlled by the polarity of the applied voltage.

When using pressure driven flow to load beads, a back pressure must be applied to the additional side channels during loading, or else the reservoirs attached to the additional side channels may be temporarily sealed. When flushing the beads from the chamber 4, a pressure may be applied to the bead supply channel 5a to flush beads out of one or more additional side channels.

When performing SPE or CEC using a multiple side channel design and electrokinetic forces, a voltage may be applied to the additional side channels to prevent leakage of sample or beads out of the trapping zone and into the side channels, substantially in the same manner as described for a single side channel in the trapping zone. When using pressure drive pumping, the side channels may have enough positive pressure applied to eliminate flow into the side channel, or else the reservoirs attached to the respective side channels can be temporarily sealed.

Dimension Guidelines

While the theoretical limits of various dimensions of a microfluidic device designed according to the present invention are not known, the inventors have adopted some general guidelines for practical purposes, which are discussed below.

It is thought that the length of the trapping zone may range anywhere from about 10 μm up to about 200 μm (using a coiled or serpentine path if necessary to allow for incorporation of such a length within the confines of a single device wafer). The trapping zone length required will be dependent upon the application and will also be limited by the forces which may be applied to achieve packing and unpacking. For example, on-chip CEC would require relatively long trapping zones, with a preferred upper limit of about 5 cm.

As to the depth of the trapping zone, sample and waste channels, a practical range is estimated to be about 400 μm to 0.25 μm . More preferably, the upper limit should be about 100 μm and the lower limit should be about 10% larger than the particle depth at a minimum.

Also, in order to reduce the likelihood of clogging, the bead delivery and bead waste channels (side channels 5, 5a 5d) preferably should be at least about 3 times deeper and three times wider than the bead diameter.

The maximum dimensions of the side channels 5, 5a-5d are also dependent upon the relative flow resistances required (i.e. the flow resistance of the side channel versus the main channel and the weirs, so as to minimize side channel backflow during use). Generally, speaking, the flow resistance of the side channels should be higher than the flow resistance of weirs to minimize the backflow problem.

The accompanying tables provide information on the calculated effect of channel and weir dimensions on the volumetric flow rates out of the trapping zone, as a function of flow channel depth, weir depth and side channel length using pressure driven flow.

16

In the tables below, what is called channel W is element 1 in FIG. 1A; what is called channel C is called element 5 in FIG. 1A; and what is called channel C' is element 3 in FIG. 1A

Correlation to FIG. 1A		Width	Length
20 μm Deep			
Element 1	Channel W	600	6,500
	Weir	280	variable
Element 5	Channel C	50	variable
Element 3	Channel C'	600	3,500
10 μm Deep			
Element 1	Channel W	580	6,500
	Weir	280	variable

The volumetric flow rates were estimated using the Navier-Stokes equation for a rectangular channel cross section and Perry's tabulated values of the effect of channel shape. The flow resistance of a channel with half width a and half depth b is given by equation 2:

$$\Delta P/U = hL/abN \quad (2)$$

where ΔP is the pressure drop along a channel segment of length L, U is the average linear flow velocity, h is the viscosity, and N is a form factor dependent upon the cross sectional ratio b/a (b<a). The factor N may be estimated from solutions to the Navier-Stokes equation for pressure driven, parabolic flow, and was tabulated by Perry in Chemical Engineer's Handbook, (3rd edition, 1950) pp 387. The goal in device design is to make the resistance of the side channel, C in the Tables, higher than the resistance of the weir and the following flow channel W, so that flow across the weir is favoured. When flow elements are in series the fluid resistance given by the right hand side of equation 1 for each segment can be added in the manner that the resistance of series electrical impedances can be added. When fluid elements are in parallel the inverse of their fluid resistance can be added to obtain the inverse of the total impedance, as is done for parallel electrical resistances. The volumetric flow rate Q, through a channel or a combination of channels is then given by equation 3.

$$Q = ab\Delta P/R_f \quad (3)$$

Where R_f is the resistance to fluid flow defined by the right hand side of equation 1, combined to gether for all channel segments as discussed above. The ratio, r, of volumetric flow rate across the weir, Q_w versus into the side channel, Q_C , $r = Q_w/Q_C$, should be large to ensure the percent of solution flowing across the weir, $\% Q_w = 1/(1+r)$, is high. This can be accomplished by using a long narrow side channel compared to a wide main channel, by increasing the depth of the weir relative to the depth of the other channels, by decreasing the depth of the side channel relative to the main channel, etc, as indicated by several calculations presented in the Tables.

TABLE

Volumetric Flow Ratios for 10 and 20 μm Deep Designs					
Channel depth	Channel C Length	Weir Depth	Weir Length	Volumetric ratio r	$\% Q_w$
20 μm	15,000	3	20	12.58	92.6
	25,000			20.85	95.4
	15,000		10	18.75	94.9
20 μm	15,000	1	20	0.687	41.1

TABLE-continued

Volumetric Flow Ratios for 10 and 20 μm Deep Designs					
Channel depth	Channel C Length	Weir Depth	Weir Length	Volumetric ratio r	% Q_W
10 μm	25,000	3	10	1.16	53.6
	15,000			1.37	57.8
	15,000		40	38.9	97.5
	25,000			63.7	98.5
10 μm	15,000	1	30	41.7	97.7
	15,000			4.14	80.5
	25,000		40	6.87	87.3
	15,000			5.83	84.3

TABLE

Fixed Device Dimensions for Calculations with a Given Etch Depth		
	Width	Length
<u>20 μm Deep</u>		
Channel W	600	6,500
Weir	280	variable
Channel C	50	variable
Channel C'	600	3,500
<u>10 μm Deep</u>		
Channel W	580	6,500
Weir	280	variable
Channel C	30	variable
Channel C'	580	3,500

Integrated Analytical Procedures

It will be appreciated that the various features of the present invention as described above may be utilized in a more complex microfluidic design.

FIG. 9 shows a multiple weir and multiple side channel design, generally referred to by reference numeral 20, in which several trapping zones are integrated, each serving a different function.

As an illustrative example, in a first trapping zone 25, formed between weirs 6a and 6b, beads loaded with an antibody to a specific protein are introduced via side channel 24 (and exit via side channel 26). A cell lysate or serum sample or other protein source is directed from a sample reservoir (not shown) and loaded into the chip via sample inlet 21 and entrance channel 38 (the sample is removed at sample outlet 22 and an eluent inlet 23 is also provided at the entrance channel. The sample is then passed into the antibody bead bed in trapping zone 25 to isolate a specific protein, while the effluent is directed towards waste outlet 27.

A chaotropic elution agent, such as an acetonitrile, water mix, is then introduced (eluent inlet 23) to elute the protein from the column and deliver it to the next trapping zone 30 (formed between weirs 6c and 6d) where it is designed by a protease enzyme immobilized on beads loaded into the zone 30 (via side channels 29, 31). The effluent at this stage would be directed towards waste outlet 32. After sufficient reaction time, a buffer is delivered (elution inlet 28, running buffer 28a, waste from bed 25) to flush the protein digest from the bed and into the next trapping zone 35 (formed between weirs 6e and 6f) with effluent delivered to waste outlet 39.

The third trapping zone 35 contains a solid phase extraction material (packed and unpacked via side channels 34, 36), allowing concentration of the digest peptides onto the bed in zone 35. An elution solvent, such as a methanol/aqueous mixture or acetonitrile/aqueous mixture is then introduced (elution inlet 33, running buffer 33a) to deliver (exit channel

37, waste 39, or collection 40) a concentrated protein digest to another location on the chip for final analysis.

Packed Bed Chip to Electrospray Mass Spectrometry Interface

5 Packed bed flow channels may according to the present invention may be interfaced to a mass spectrometer via an electrospray coupler 41, as illustrated in FIG. 10. The packed bed 4 may perform an enzyme digestion of a protein, affinity purification and pre-concentration of a specific chemical or protein, solid phase extraction concentration enhancement, or capillary electrochromatographic separation, or any combination of these and other steps, prior to electrospray introduction in to a mass spectrometer. The chip to electrospray interface may be made using any method that provides a less than 100 nL dead volume, preferably less than 1 nL and most preferably less than 100 pL dead volume at the coupling region. A method such as that described by Wang et al, or Karger can be used to create the interface [Bings, N. H.; Wang, C.; Skinner, C. D.; Colyer, C. L.; Thibeault, P.; Harrison, D. J. Anal. Chem. 71 (1999) 3292-3296. Zhang, B.; Liu, H.; Karger, B. L.; Foret, F. Anal. Chem 71 (1999) 3258-3264].

While the present invention has been described by reference to various preferred embodiments, it will be understood that obvious changes may be made and equivalents substituted without departing from the true spirit and scope of the invention which is set out in the following claims.

We claim:

1. A microfluidic analysis system, comprising:
 - (a) a substantially planar substrate having an upper surface;
 - (b) at least one main channel formed into said upper surface, said main channel having first and second ends and a defined direction of flow in use;
 - (c) a cover plate arranged over said planar substrate, said cover plate closing off said channel from above;
 - (d) a first weir formed across said main channel and between said first and second ends of said channel, said first weir providing at least one flow gap to allow, in use, at least some fluid to flow past said first weir while trapping packing material having constituent particles that are generally larger than said flow gap; [and]
 - (e) at least one side channel formed into said planar substrate, said side channel being connected at a first end to said main channel at a location upstream from said first weir, and at a second end to a reservoir, said side channel providing a higher flow resistance than said main channel; and
 - [(e)] (f) a second weir located upstream from said connected first end of said side channel, said first and second weirs forming a chamber therebetween, said second weir providing at least one flow gap to allow, in use, at least some fluid to flow past said second weir while trapping said packing material within said chamber.

2. The microfluidic analysis system claimed in claim 1, further comprising at least one side channel formed into said planar substrate, said side channel being connected at a first end to said main channel at a location upstream from said first weir, and at a second end to a reservoir, said side channel providing a higher flow resistance than said main channel.]

3. The microfluidic analysis system claimed in claim [2] 1, wherein, each side channel connection to said main channel is provided with a hook structure whereby, in use, packing material is sprayed into said chamber to facilitate even packing.

4. The microfluidic analysis system claimed in claim 3, wherein, said hook structure at least partially obstructs direct

19

line-of-sight entry of packing material from said side channel into said chamber and forms a chamber mouth to one side of said hook structure.

5. The microfluidic analysis system claimed in claim 1, wherein, said flow gaps comprise a generally uniform gap between said cover plate and the top of said weirs.

6. The microfluidic analysis system claimed in claim 1, wherein, said flow gaps comprise a plurality of substantially vertical gaps in said weirs.

7. The microfluidic analysis system claimed in any previous claim, wherein, said system is formed entirely on a single microfluidic chip.

8. A method of creating a packed reactor bed in the microfluidic analysis system claimed in claim [2] 1, said method comprising, effecting a pressure driven flow by providing a relatively high pressure at said second end of said main channel and at said reservoir, said reservoir containing packing material, and providing a relatively low pressure at said first end of said main channel, whereby, packing material flows from said reservoir into said main channel and is trapped against said first weir.

9. The method as claimed in claim 8, wherein, packing material is removed from the said main channel by providing relatively high pressure at said first and second ends of said main channel while providing relatively low pressure at said reservoir.

10. A method of packing the chamber in the microfluidic analysis system claimed in claim [2] 1, said method comprising, providing a non-conductive substrate and effecting an electrokinetic flow by applying a relatively high voltage at said reservoir, said reservoir containing packing material, and providing relatively low voltages at said first and second ends of said main channel, whereby, packing material flows from said reservoir into said chamber and is trapped by said first and second weirs.

11. The method as claimed in claim 10, wherein, packing material may be removed from the chamber by reversing said electrokinetic flow.

12. A method of packing the chamber in the microfluidic analysis system claimed in claim [2] 1, said method comprising, effecting a pressure driven flow by providing a relatively high pressure at said reservoir, said reservoir containing packing material, and providing relatively low pressure at said first and second main reservoirs, whereby, packing material flows from said packing material reservoir into said chamber and is trapped by said first and second weirs.

13. The method as claimed in claim 12, wherein, packing material may be removed from the chamber by reversing said pressure driven flow.

14. A method of packing the chamber in the microfluidic analysis system claimed in claim [13] 1, said method comprising, providing magnetically charged packing material, and effecting a magnetically driven flow by providing a magnetically attractive force in the chamber, whereby, the packing material enters the chamber and is trapped by said first and second weirs.

15. The method as claimed in claim 14, wherein, packing material may be removed from the chamber by reversing said magnetic force in said chamber.

16. A method of creating a packed reactor bed in a microfluidic analysis system comprising:

- (a) a substantially planar substrate having an upper surface;
- (b) at least one main channel formed into said upper surface, said main channel having first and second ends and a defined direction of flow in use;
- (c) a cover plate arranged over said planar substrate, said cover plate closing off said channel from above;

20

(d) a first weir formed across said main channel and between said first and second ends of said channel, said first weir providing at least one flow gap to allow, in use, at least some fluid to flow past said first weir while trapping packing material having constituent particles that are generally larger than said flow gap; and

(e) at least one side channel formed into said planar substrate, said side channel being connected at a first end to said main channel at a location upstream from said first weir, and at a second end to a reservoir, said side channel providing a higher flow resistance than said main channel,

said method comprising, providing a non-conductive substrate and effecting an electrokinetic flow by applying a relatively high voltage at said second end of said main channel and at said reservoir, said reservoir containing packing material, and providing a relatively low voltage at said first end of said main channel, whereby, packing material flows from said reservoir into said main channel and is trapped against said first weir.

17. The method as claimed in claim 16, wherein, packing material is removed from said main channel by providing a relatively high voltage at said first and second ends of said main channel while providing a relatively low voltage at said reservoir.

18. A method of treating a sample within a microfluidic analysis system, comprising the steps of:

- (a) providing a main channel having a trapping zone suitable for trapping packing material;
- (b) providing a first weir positioned at a first end of said trapping zone, wherein said first weir has at least one flow gap to allow, in use, at least some fluid to flow past said first weir while trapping said packing material within said zone;
- (c) providing a second weir positioned at a second end of said trapping zone, wherein said second weir has at least one flow gap to allow, in use, at least some fluid to flow past said second weir while trapping said packing material within said zone;
- (d) providing a slurry of a reagent treated packing material prepared in a solution having a predetermined composition of a solvent;
- (e) providing a flow channel having a first end connected to said trapping zone, wherein said first end is positioned between said first weir and said second weir;
- (f) inducing a flow of said packing material into said trapping zone through said flow channel so as to load said trapping zone and form a packed bed of said packing material; and
- (g) flowing a sample containing analytes through said packed bed, said reagent treating the sample, whereby the sample leaving the trapping zone has an altered analyte composition.

19. The method claimed in claim 18, further comprising the step of:

- (h) adjusting the composition of the solvent, so as to affect the aggregation of said packing material and the stabilization of the packed bed.

20. The method claimed in claim 18 wherein, step (d) comprises providing packing material comprising porous beads.

21. The method claimed in claim 20, wherein said porous beads are selected to have a diameter in the range from about 0.7 to about 10.0 μm .

21

22. The method claimed in claim **21**, wherein said solvent is acetonitrile, and step (h) comprises adjusting the concentration level to less than about 50% to stabilize the packed bed.

23. The method claimed in claim **22** further including the steps of adjusting the concentration level to above 50% to destabilize the packed bed, and reversing the flow in step (f) so as to unload said trapping zone.

24. The method claimed in claim **23**, further including the step of repeating step (f) so as to reload said trapping zone, and readjusting the concentration level to restabilize the packed bed.

25. The method claimed in claim **21**, wherein said solvent is acetonitrile, and step (h) comprises adjusting the concentration level to less than about 30% to stabilize the packed bed.

26. The method claimed in claim **20**, wherein said porous beads are selected to have a diameter in the range from about 1.5 to about 4.0 μm .

27. The method claimed in claim **18**, further comprising the steps of:

before step (f), adding a neutral surfactant to said packing material so as to inhibit aggregation; and

22

after step (f), removing the neutral surfactant to promote aggregation.

28. The method claimed in claim **18**, wherein step (f) comprises applying a fluid force to induce the flow of said packing material.

29. The method claimed in claim **18**, wherein said packing material comprises at least some electrically charged particles and step (f) comprises applying a voltage potential to induce the flow of said packing material.

30. The method claimed in claim **18**, wherein said packing material comprises at least some particles susceptible to a magnetic field and step (f) comprises applying a magnetic field to induce the flow of said packing material.

31. The method claimed in any one of claims **18, 19, 20, 21, 26, 23, 24, 27, 28, 29** and **30**, including the step of providing a hook structure at the connection point between said flow channel and said trapping zone, so as to prevent direct line-of-sight entry of said packing material, thereby to promote even packing.

* * * * *