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(54) Title: SUPERPARAMAGNETIC NANOPARTICLES BASED ON IRON OXIDES WITH MODIFIED SURFACE, METHOD OF THEIR PREPARATION AND APPLICATION

(57) Abstract: The subject of the invention is superparamagnetic nanoparticle probes based on iron oxides, to advantage magnetite or maghemite, with modified surface, coated with mono-, di- or polysaccharides from the group including D-arabinose, D-glucose, D-galactose, D-mannose, lactose, maltose, dextrans and dextrans, or with amino acids or poly(amino acid)s from the group including alanine, glycine, glutamine, asparagine, histidine, arginine, L-lysine, aspartic and glutamic acid or with synthetic polymers based on (meth)acrylic acid and their derivatives selected from the group containing poly(N,N-dimethylacrylamide), poly(N,N-dimethylmethacrylamide), poly(N,N-diethylacrylamide), poly(N,N-diethylmethacrylamide), poly(N-isopropylacrylamide), poly(N-isopropylmethacrylamide), which form a colloid consisting of particles with narrow distribution with polydispersity index smaller than 1.3, the average size of which amounts to 0.5-30 nm, to advantage 1-10 nm, the iron content is 70- 99.9 wt.%, to advantage 90 wt.%, the modification agent content 0.1-30 wt.%, to advantage 10 wt.%. The particles of size smaller than 2 nm with polydispersity index smaller than 1.1 can be obtained by a modified method of preparation. Superparamagnetic nanoparticle probes according to the invention are prepared by pre- precipitation of colloidal Fe(OH)₃ by the treatment of aqueous 0.1-0.2M solution of Fe(III) salt, to advantage FeCl₃, with less than an equimolar amount of NH₄OH, at 21°C, under sonication, to which a solution of a Fe(II) salt, to advantage FeCl₂, is added in the mole ratio Fe(III)/Fe(II) = 2 under sonication and the mixture is poured into five- to tenfold, to advantage eightfold, molar excess of 0.5M NH₄OH. The mixture is left aging for 0-30 min, to advantage 15 min, and then the precipitate is repeatedly, to advantage 7-10 times, magnetically separated and washed with deionized water. Then 1-3 fold amount, to advantage 1.5 fold amount, relative to the amount of magnetite, of 0.1 M aqueous solution of sodium citrate is added and then, drop wise, 1-3 fold amount, to advantage 1.5 fold amount, relative to the amount of magnetite, of 0.7 M aqueous solution of sodium hypochlorite. The precipitate is repeatedly, to advantage 7-10 times, washed with deionized water under the formation of colloidal maghemite to which, after dilution, is added drop wise, to advantage under 5 -min sonication, an aqueous solution of a modification agent, in the weight ratio modification agent/iron oxide = 0.1-10, to advantage 0.2 for amino acids and poly(amino acid)s and 5 for saccharides. The particles smaller than 2 nm. with polydispersity index smaller than 1.1 are prepared by mixing at 21°C 1 volume part of 10-60 wt. %, to advantage 50 wt.%, of an aqueous solution of a saccharide, disaccharide or polysaccharide, such as D-arabinose, D-glucose, D-galactose, D-mannose, lactose, maltose, dextran and dextrans, and 1 volume part of aqueous solution of a Fe(II) and Fe(III) salt, to advantage FeCl₂ and FeCl₃, where the molar ratio Fe(III)/Fe(II) = 2. A 5-15 %, to advantage 7.5 %, solution of NH₄OH is added until pH 12 is attained and



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A. CLASSIFICATION OF SUBJECT MATTER

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C09C C01G A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 4 452 773 A (MOLDAY ROBERT S [CA]) 5 June 1984 (1984-06-05)	1,5-7
Y	column 3, line 1 - line 22 column 5, line 12 - line 28 column 8, line 33 - line 50 column 11, line 8 - line 17	4
A	US 4 827 945 A (GROMAN ERNEST V [US] ET AL) 9 May 1989 (1989-05-09) column 4, line 60 - column 5, line 48	1,5-7
X	US 5 492 814 A (WEISSLEDER RALPH [US]) 20 February 1996 (1996-02-20) column 5, line 5 - column 7, line 40; examples 1,9,14	1,2,5-7
Y	column 14, line 42 - line 53 column 18, line 46 - line 67	4
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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	EP 0 516 252 A2 (DIAGNOSTIKFORSCHUNG INST [DE]) 2 December 1992 (1992-12-02) page 8, line 16 - line 22 -----	4
Y	WO 91/02811 A (IMMUNICON CORP [US]) 7 March 1991 (1991-03-07) page 11, line 20 - page 12, line 25 -----	4
Y	US 4 101 435 A (HASEGAWA MASAKATSU ET AL) 18 July 1978 (1978-07-18)	4
A	column 2, line 37 - line 50; example 1 -----	3
A	DE 26 42 383 A1 (SHERRITT GORDON MINES LTD) 31 March 1977 (1977-03-31) page 9, line 19 - line 28; example 1 -----	3
X	US 2005/271593 A1 (YEH CHEN-SHENG [TW] ET AL) 8 December 2005 (2005-12-08) paragraph [0007] - paragraph [0016] -----	1,5-7
X	WO 97/35200 A (SILICA GEL GMBH [DE]; PILGRIMM HERBERT [DE]) 25 September 1997 (1997-09-25) page 5, line 9 - line 31 example 1 page 6, line 16 - line 31 page 9, line 15 - line 20 examples 1,4 -----	1,5-7

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/CZ2007/000012

Patent document cited in search report		Publication date		Patent family member(s)	Publication date
US 4452773	A	05-06-1984	CA	1217934 A1	17-02-1987
US 4827945	A	09-05-1989	AT	143604 T	15-10-1996
			CA	1301063 C	19-05-1992
			DE	3751918 D1	07-11-1996
			DE	3751918 T2	20-03-1997
			EP	0275285 A1	27-07-1988
			JP	1500196 T	26-01-1989
			WO	8800060 A1	14-01-1988
US 5492814	A	20-02-1996	MX	9100135 A1	28-02-1992
EP 0516252	A2	02-12-1992	AT	156021 T	15-08-1997
			AU	653220 B2	22-09-1994
			AU	1618492 A	03-12-1992
			CA	2068632 A1	29-11-1992
			DE	4117782 A1	03-12-1992
			DK	516252 T3	25-08-1997
			ES	2106134 T3	01-11-1997
			GR	3024898 T3	30-01-1998
			IE	921595 A1	02-12-1992
			IL	101929 A	26-08-1994
			JP	3306810 B2	24-07-2002
			JP	7122410 A	12-05-1995
			NO	921767 A	30-11-1992
			NZ	242669 A	28-08-1995
			US	5427767 A	27-06-1995
			ZA	9203299 A	30-12-1992
WO 9102811	A	07-03-1991	AT	137804 T	15-05-1996
			AU	6340390 A	03-04-1991
			CA	2060182 A1	23-02-1991
			DE	69026949 D1	13-06-1996
			DE	69026949 T2	28-11-1996
			EP	0489119 A1	10-06-1992
			JP	5503188 T	27-05-1993
US 4101435	A	18-07-1978	JP	1240649 C	26-11-1984
			JP	51151320 A	25-12-1976
			JP	59013521 B	30-03-1984
DE 2642383	A1	31-03-1977	CA	1066483 A1	20-11-1979
			JP	52056010 A	09-05-1977
US 2005271593	A1	08-12-2005	DE	102004035803 A1	03-03-2005
			TW	588018 B	21-05-2004
WO 9735200	A	25-09-1997	AT	212442 T	15-02-2002
			DE	19612001 A1	25-09-1997
			EP	0888545 A1	07-01-1999
			ES	2171930 T3	16-09-2002
			JP	2000507197 T	13-06-2000
			US	6638494 B1	28-10-2003