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Synthesis, physico-chemical and biological study of trialkylsiloxyalkyl amine coated iron oxide/oleic acid magnetic nanoparticles for the treatment of cancer

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New original water-soluble magnetic nanoparticles based on natural components, magnetite–oleic acid–biologically active silyl modified alkanolamine, were synthesized. Physico-chemical characterization, i.e. magnetic properties, concentration of magnetite, size of iron oxide core, of the nanoparticles synthesized and the corresponding magnetic fluids obtained, was carried out. Magnetic fluids were screened for *in vitro* cytotoxicity concerning human fibrosarcoma (HT-1080), mouse hepatoma (MG-22A) monolayer tumour cell lines and normal mouse fibroblasts (NIH 3T3). They possess low or moderate cytotoxic effects, are non-toxic, exhibit high NO-induction ability and strongly change tumour cell morphology. **Copyright © 2008 John Wiley & Sons, Ltd.**

Keywords: nanoparticles; magnetosomes; magnetic fluids; iron oxide; organosilicon compounds; alkanolamines; drug research; cytotoxicity

Introduction

In the last few decades magnetic nanoparticles have had a large impact in biomedicine, including drug targeting, diagnostics, immunoassays, molecular biology, DNA purification, cell separation and purification and hyperthermia therapy.[1–7] They can be coated with different biological molecules in order to interact with the biological entity of interest, but possessing magnetic properties they can be manipulated by an external magnetic field. When considering the coating materials for drug delivery applications, it is usually required that particles have sufficiently hydrophilic surfaces and relatively small sizes so they can evade the reticuloendothelial system.[8] Therefore, creation of stable, small molecular-sized magnetic nanoparticles for biomedical applications is very important among the numerous existing large molecular ones.

Some years ago our research team started the targeted searching of medical remedies based on iron oxide magnetic fluids (MFs), functionalized with a wide spectrum of low-molecular-weight biologically active compounds with different kinds of biological activities and potentially prolonged action. The objective of our investigation was to study the uptake of nano-sized iron oxide magnetic particles into cells and their modification for targeting specific cells. We designed biocompatible magnetic nanoparticles, assembling a final product composed of a magnetic core, a biocompatible shell and biologically active molecules anchored to the surface, allowing a two-fold anticancer action capable of combining the therapeutic effect based on targeted drug-delivery with hyperthermia for the treatment of widespread disease. A methodological approach allowing the synthesis of the nanoparticles has been developed. The first model magnetic nanoparticles, bearing absorbed cytotoxic organosilicon choline and colamine derivatives, to be accepted as silyl prodrugs, capable to easily penetrating the blood–brain barrier and possessing prolongated action, have been synthesized,[9] and water-based MFs containing model cytotoxic magnetosomes bearing *n*-decyldimethyl(β-dimethylaminoethoxy)silane methiodide were obtained.[10]

In continuation of our research, we present the synthesis and investigation of new highly dispersed magnetite nanoparticle systems with average particle size of 10 nm embedded in different chemical biocompatible environments.

In this contribution we report on the application of the developed methodological approach to the synthesis of new double-coated iron oxide magnetic nanoparticles containing chemisorbed oleic acid covered with cytotoxic trialkylsiloxyalkyl alkanolamines possessing antitumour properties and affecting central nervous system diseases, in order to evaluate the efficacy of these nanostructures as antitumour agents. Monolayer oleic acid-modified iron oxide nanoparticles provide the basis for further surface modification and functionalization with biocompatible layers of trialkylsiloxyalkyl amine bioactive molecules containing

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$[R_3SiOCH_2CH_2NMe_3]^+ I^-$

A1-A5

R_3: $C_{10}H_{21}Me_2$ (**A1**), $(C_{10}H_{21})_2Me$ (**A2**), $C_{16}H_{33}Me_2$ (**A3**), $C_8H_{17}Me_2$ (**A4**), $EtBu_2$ (**A5**)

Figure 1. General formula of silylalkanolamines **A1**–**A5**.

long lipophilic tails, which are able to deepen inside the oleic acid shell, forming liposome-like structures.

Physico-chemical characterization (magnetic properties, particle size, magnetite concentration) and biological investigation (cellular uptake, antitumour activity) of the nanoparticles synthesized and the corresponding MFs obtained were carried out. MFs prepared were screened for *in vitro* cytotoxicity on monolayer HT-1080 (human fibrosarcoma), MG-22A (mouse hepatoma) tumour cell lines and normal mouse fibroblasts (NIH 3T3).

Experimental

Chemicals and instrumentation

^1H, ^{13}C and ^{29}Si NMR spectra for silylalkanolamines methiodides were obtained on a Varian Mercury 200 spectrometer at 200, 50 and 40 MHz, respectively, at 303 K with $CDCl_3$ as a solvent and internal standard ($\delta = 7.25$ ppm for $CHCl_3$). Elemental analyses (C, H, N) of silylalkanolamine methiodides and nanoparticles were performed on a Carlo Erba 1108 elemental analyzer. Elemental analysis results for silylalkanolamines methiodides agreed with calculated values. Melting points for silylalkanolamines methiodides were determined on a Boetius melting point apparatus and were taken uncorrected. Solvents and reagents were purchased from Acros and Aldrich. All solvents used were freshly dried using standard techniques and all glassware was oven-dried. The dependence of magnetization on external magnetic field for the samples was determined using a magnetometer with vibrating sample (VSM), Lake Shore Cryotronics Inc., model 7404.

Synthesis of silylalkanolamines

n-Decyldimethyl-, *n*-didecylmethyl-, *n*-hexadecyldimethyl-, *n*-octyldimethyl- and ethyldi-*n*-butyl(β-dimethylaminoethoxy) silane methiodides A1–A5 of the general formula presented in Fig. 1, used as surfactants for magnetic nanoparticles precoated with oleic acid, were synthesized by reaction of alkanolamine with corresponding hydrosilane in the presence of metallic sodium and subsequent methylation by methyl iodide and characterized by NMR spectroscopy method, elemental analyses and melting point according to Zablotskaya *et al*.[11]

n-Decyldimethyl(β-dimethylaminoethoxy)silane methiodide (**A1**). ^1H NMR, δ (ppm): 0.15 (6H, s, $SiCH_3$), 0.44 (2H, m, $SiCH_2$), 0.98 (3H, t, $J = 6$ Hz, CH_3), 1.36 (16H, m, CH_2), 3.51 (9H, s, N^+CH_3), 3.85 (2H, t, $J = 4.6$, OCH_2), 4.05 (2H, m, N^+CH_2). ^{13}C NMR ($CDCl_3$), δ (ppm): −2.43 (CH_3Si), 13.98 (CH_3–C), 15.76 (CH_2Si), 22.55, 22.93, 29.46, 29,49, 31.78 and 33.21 (C–CH_2–C), 54.83 (CH_3–N^+), 57.22 (CH_2–N^+), 67.57 and (CH_2O). ^{29}Si NMR ($CDCl_3$), δ (ppm): 22.46 [*n*-$C_{10}H_{21}(CH_3)_2Si$–O].

n-Didecylmethyl(β-dimethylaminoethoxy)silane methiodide (**A2**). ^1H NMR, δ (ppm): 0.09 (3H, s, $SiCH_3$), 0.61 (4H, m, $SiCH_2$), 0.90 (6H, m, CH_3), 1.45 (32H, m, CH_2), 3.51 (9H, s, N^+CH_3), 3.82 (2H, m, OCH_2), 4.10 (2H, m, N^+CH_2). ^{13}C NMR ($CDCl_3$), δ (ppm): −4.16 (CH_3Si), 14.03 (CH_3–C), 14.47 (CH_2Si), 22.59, 23.01, 29.20, 29.25, 29.50, 29.55, 31.82 and 33.36 (C–CH_2–C), 54.87 (CH_3N^+), 57.35 (CH_2N^+), 67.72 (CH_2O). ^{29}Si NMR ($CDCl_3$), δ (ppm): 22.28 [CH_3(*n*-$C_{10}H_{21})_2Si$–O].

n-Hexadecyldimethyl(β-dimethylaminoethoxy)silane methiodide (**A3**). ^1H NMR, δ (ppm): 0.13 (6H, s, $SiCH_3$), 0.59 (2H, t, $J = 7$ Hz, $SiCH_2$), 0.87 (3H, t, $J = 6$ Hz, CH_3), 1.25 (28H, s, CH_2), 3.51 (9H, s, N^+CH_3), 3.88 (2H, t, $J = 5$ Hz, OCH_2), 4.06 (2H, m, N^+CH_2). ^{13}C NMR ($CDCl_3$), δ (ppm): −2.44 (CH_3Si), 13.65 (CH_3–C), 15.77 (CH_2Si), 22.06, 22.95, 29.22, 29.49, 29,55, 29.60, 31.82 and 33.28 (C–CH_2–C), 54.83 (CH_3N^+), 57.22 (CH_2N^+) and 67.37 (CH_2O). ^{29}Si NMR ($CDCl_3$), δ (ppm): 22.49 [*n*-$C_{16}H_{33}(CH_3)_2Si$–O].

n-Octyldimethyl(β-dimethylaminoethoxy)silane methiodide (**A4**). ^1H NMR, δ (ppm): 0.12 (6H, s, $SiCH_3$), 0.58 (2H, t, $J = 8$ Hz, $SiCH_2$), 0.86 (3H, t, $J = 6$ Hz, CH_3), 1.25 (12H, bs, CH_2), 3.61 (9H, s, N^+CH_3), 3.87 (2H, m, OCH_2), 4.04 (2H, m, N^+CH_2). ^{13}C NMR ($CDCl_3$), δ (ppm): −2.52 (CH_3Si), 13.90 (CH_3–C), 15.65 (CH_2Si), 22.43, 22.81, 29.00, 31.65 and 33.11 (C–CH_2–C), 54.73 (CH_3N^+), 57.13 (CH_2N^+), 67.44 (CH_2O). ^{29}Si NMR ($CDCl_3$), δ (ppm): 22.37 [*n*-$C_8H_{17}(CH_3)_2Si$–O].

Ethyldi-*n*-butyl(β-dimethylaminoethoxy)silane methiodide (**A5**). ^1H NMR, δ (ppm): 0.61 (6H, m, $SiCH_2$), 0.91 (13H, m, CH_3 and CH_2), 1.74 (4H, m, CH_2), 3.53 (9H, s, N^+CH_3), 3.87 (2H, m, OCH_2), 4.07 (2H, m, N^+CH_2). ^{13}C NMR ($CDCl_3$), δ (ppm): 6.04 and 6.84 (CH_2Si), 23.68, 24.01, 26.16 (CH_3–C and C–CH_2–C), 54.76 (CH_3N^+), 57.32 (CH_2N^+), 67.80 (CH_2O). ^{29}Si NMR ($CDCl_3$), δ (ppm): 21.25 [C_2H_5(*n*-$C_4H_9)_2Si$–O].

General procedure for synthesis of nanoparticles

Magnetic particles (Fe_3O_4) were obtained by wet synthesis. Initial toluene-based MF **1** was synthesized by reaction of magnetic particles obtained with oleic acid (OA) according to Bibik.[12] Magnetic powder **2a** was prepared from initial MF **1** by treating with acetone. Magnetic powders **3**–**6** were prepared by shaking MF **1** with corresponding silylalkanolamine (2.3 mol of A1–A5 per 1 mol of OA) and consecutively treating with acetone. A2–A5 were added in the same quantity as in the case of the water-based MF **17** containing A1.[10] Magnetic powders **3**–**6** were treated with water in the presence of ammonium hydroxide to produce water-based MFs **7**–**10** from A2–A5, correspondingly. Water soluble powders **11**–**14** were obtained by solvent evaporation from the corresponding MFs **7**–**10**. The scheme of synthesis of magnetic iron oxide-based samples is presented in Fig. 2.

Magnetic nanoparticles synthesized were characterized by molar ratio of components calculated on the basis of element analysis data. We estimated the changes in concentration, magnetization and size of nanoparticles during the process of sedimentation in dependence on the polarity and the amount of the solvent used. For this purpose 7 ml of acetone, ethyl acetate or ethanol were added to 1 ml of MF **1** to give pastes **2b**–**2d**, or 5 ml of the solvent to 1.5 ml of MF **1** to give pastes **2e**–**2g**, which were converted to MFs **16e**–**16g** by addition of 1.5 ml of toluene. The powdery samples **15a** and **15b** were obtained from 3 ml of the toluene-based MF, containing A1 by its treatment with 40 ml of acetone or ethanol.

Magnetic properties of ferromagnetic samples

The method of magnetogranulometry was used for the determination of the diameter of iron oxide magnetic core and magnetic

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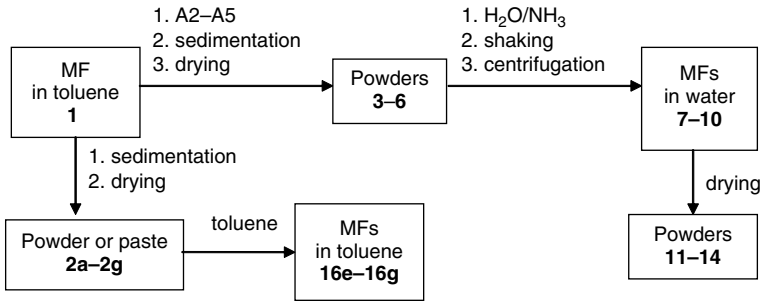

Figure 2. Scheme of preparation of magnetic iron oxide based samples.

properties of the prepared nanoparticles. Magnetic properties of magnetic fluids were studied in solutions without separating the carrier fluid. The special glass container with MF was placed in the measuring system of the magnetometer. Magnetization curves were recorded at different stages of the material treatment for the monitoring magnetite concentration and its condition as well. As a rule, measurements were carried out at room temperature for fields up to 10 KOe. The magnetization curves were used for the magnetogranulometry analysis[13] to find full spontaneous magnetization and magnetic moment of magnetite particles in the sample from which the magnetite concentration and the particle sizes in the sample were calculated. To analyze the weak magnetic samples, the matrix (water or toluene) at the same holder was measured separately. The matrix magnetization was subtracted from full magnetization.

Cytotoxicity assays

Monolayer tumour cell lines MG-22A (mouse hepatoma) and HT-1080 (human fibrosarcoma) and normal mouse fibroblasts (NIH 3T3) were cultivated for 72 h in DMEM (Dulbecco's modified Eagle's medium) standard medium (Sigma) without an indicator and antibiotics.[14] Tumour cell lines were taken from the European Collection of Cell Culture (ECACC). After the ampoule was thawed, not more than four passages were performed. The control cells and cells with tested substances in the range of $2-5 \times 10^4$ cells/ml concentration (depending on line nature) were placed in separate 96-well plates. The volume of each plate was 200 µl. MFs were diluted with water and added in wells. The samples before addition were kept in a water bath at 80 °C for 1 h. Control cells were treated in the same manner only in the absence of test compounds. The plates were incubated for 72 h at 37 °C and 5% CO_2. The number of surviving cells was determined using tri(4-dimethylaminophenyl)methyl chloride (crystal violet: CV) or 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) coloration, which was assayed by multiscan spectrophotometer. The quantity of alive cells on the control plate was taken in the calculations as 100%.[14,15]

The LC_{50} was calculated using Graph Pad Prism® 3.0 program, $r < 0.05$. LD_{50} values were calculated based on the data obtained for normal cells NIH 3T3 using a special program (Graph Pad Prism® 3.0 program). According to the protocols of Committee on the Validation of Alternative Methods (ICCVAM) and National Toxicology Program (NTP), these data are alternatives to LD_{50} values, obtained in *in vivo* tests.[16]

Table 1. Physico-chemical properties of magnetic powders **2a** and **3–6**

No	σ (emu/g)	C (%)	Size (nm)		
			d	d_{min}	d_{max}
2a	6.4	7.0	8.6	4.6	12.3
3	5.9	6.4	8.6	5.4	12.3
4	6.9	7.5	8.6	5.8	12.1
5	4.85	5.3	9.1	6.4	12.1
6	5.92	6.4	8.9	6.4	11.9

Results and Discussion

Physico-chemical properties, such as magnetization, magnetite concentration and particle diameter, of the prepared powdery and liquid magnetic samples were studied by the method of magnetogranulometry. Earlier we used X-ray line profile broadening analysis for crystallite size determination. The use of method of magnetogranulometry was justified by the fact that the difference between the values obtained by these two methods was found to be insignificant.[10] The magnetogranulometry gave the particles size distribution. We used the most expected particle size at the distribution as the particle diameter. For distribution width estimation we directly used d_{min} and d_{max} at level 0.5 from the distribution density maximum. The magnetization curve of MF **8** is presented in Fig. 3.

The results of physico-chemical characterization of magnetic powders **2a** and **3–6** are presented in Table 1, and those of MFs **7–10** in Table 2.

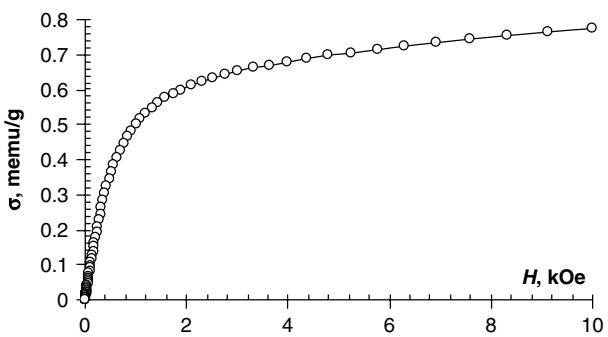

Figure 3. Magnetization curve of MF **8** (magnetization of water is subtracted).

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Table 2. Physico-chemical properties of water-based magnetic fluids **7**–**10**

No.	σ (emu/g)	C (%)	Size (nm) d	d_{min}	d_{max}
7	0.0004	0.0004	8.5	5.6	14.0
8	0.0007	0.0007	8.4	7.4	9.5
9	0.0019	0.0021	10.2	6.1	16.2
10	0.0031	0.0033	7.6	4.7	15.6

Table 3. Yield and physico-chemical characterization of water-soluble magnetic powders 11–14

No.	Yield (%)	σ (emu/g)	C (%)
11	16	0.126	0.13
12	44	0.084	0.09
13	27	0.131	0.15
14	9	0.857	0.93

Table 4. Physico-chemical properties of magnetite-based samples **1**, **2b**–**2g**, **15a**, **15b**, **16e**–**16g**

No.	Solvent	Physical state	σ (emu/g)	C (%)	Size (nm) d	d_{min}	d_{max}
1[a]	–	Solution	0.52	0.56	8.4	4.0	15.2
2b	Acetone	Paste	4.3	4.7	8.8	6.1	12.7
2c	Ethyl acetate	Paste	4.7	5.1	8.1	5.4	12.8
2d	Ethanol	Paste	4.1	4.5	8.5	5.3	13.3
2e	Acetone	Paste	3.6	3.9	7.3	5.7	12.2
2f	Ethyl acetate	Paste	4.2	4.6	8.5	5.8	12.3
2g	Ethanol	Paste	3.8	3.8	8.1	5.5	12.4
15a[b]	Acetone	Powder	4.8	5.3	8.3	5.3	12.0
15b[b]	Ethanol	Powder	5.9	6.4	8.5	5.5	12.0
16e	–	Solution[c]	0.23	0.25	6.7	5.5	10.2
16f	–	Solution[c]	0.22	0.24	6.5	5.4	12.1
16g	–	Solution[c]	0.25	0.27	7.5	6.1	10.7

[a] Initial magnetic fluid in toluene.
[b] Powders containing **A1.**
[c] Toluene solutions of **2e**–**2g.**

Table 5. *In vitro* cell cytotoxicity and intracellular NO generation caused by water-based magnetic fluids **7**–**10, 17**[a] and oleic acid (OA)

No.	HT-1080 LC_{50}[b] CV	HT-1080 LC_{50}[c] MTT	HT-1080 NO[d] CV	MG-22A LC_{50}[b] CV	MG-22A LC_{50}[c] MTT	MG-22A NO[d] CV	NIH 3T3 LC_{50}[e] NR	NIH 3T3 LD_{50}, mg/kg
7	215	32	69	235	309	350	593	3522
8	108	49	800	107	97	700	975	4208
9	209	137	125	121	114	1050	637	3291
10	120	24	750	88	143	650	527	3020
17[a][10]	159	98	1100	88	73	1200	1115	4240
OA[10]	53	48	44	34	38	213	215	1045

[a] Contains silylalkanolamine **A1.**
[b] Concentration of nanoparticles (μg/ml) providing 50% cell killing effect (CV, coloration).
[c] Concentration of nanoparticles (μg/ml) providing 50% cell killing effect (MTT, coloration).
[d] NO generation (CV: coloration), determined according to Fast *et al.*[15]
[e] Concentration of nanoparticles (μg/ml) providing 50% cell killing effect (NR, coloration), $r < 0.05$.

Magnetization (σ) for powders **2a** and **3**–**6** were 4.9–6.9 emu/g, magnetite concentration (C) 5.3–7.5% and the size of magnetic core 8.6–9.1 nm. The same parameters for MFs **7**–**10** were 0.0004–0.031 emu/g, 0.0004–0.033% and 7.6–10.2 nm, respectively.

The sorption capacity of A2–A5 on the surface of oleic acid coated magnetite was low and was equal to $\leq$0.05 mol per 1 mol of oleic acid. According to the data on magnetization obtained for the fluids **7**–**10**, the maximal magnetite concentration (68–100%) had already been obtained after the first treatment of the corresponding powder during three-fold repeated treatments of powdery samples **3**–**6** with water.

The yield of water-soluble powdery samples **11**–**14**, determined as a percentage of the amount of powders **3**–**6**, obtained from 3 ml of initial MF **1**, was 9–44% and depended on the nature of silylalkanolamine used.

The magnitude of magnetization of the powders and the concentration of magnetite, which varied from 0.084 to 0.86 emu/g, and from 0.09 to 0.93% respectively, were calculated on the basis of the same parameters obtained for water solutions. The yield and physico-chemical characterization data of magnetic powders **11**–**14** are presented in Table 3.

To characterize water-based MFs **7**–**10**, the molar ratio of components in the corresponding water soluble powders **11**–**14** was calculated on the basis of element analysis data. The molar ratio Fe_3O_4:OA in the original nanoparticles was determined as 2.7 : 1. This ratio and the molecular weight of silylalkanolamine were used to determine the molar ratio of components of nanoparticles **11**–**14**. It was about 2.7 : 1:0.25–0.65 (Fe_3O_4:OA : A2–A5), and was found to be 2.7 : 1:0.65 for powder **11,** 2.7 : 1:0.25 for powder **12**, 2.7 : 1:0.6 for powder **13** and 2.7 : 1:0.3 for powder **14.** In all cases the silylalkanolamine content in water-soluble powders increased in comparison with powders **3**–**6**. The enforced purification of double-coated nanoparticles due to their increased water solubility in comparison with mono-coated nanoparticles could be the reason for this.[10].

The influence of solvent nature (acetone, ethylacetate and ethanol) and its volume upon the changes in concentration, magnetization and size of nanoparticles as a consequence of the repeated sedimentation process has been studied. There was no significant influence of solvent polarity on the process of nanoparticle sedimentation.[17] Magnetic properties recovered for MFs **16e**–**16g** obtained after redissolving sediments **2e**–**2g** were 42–48% of initial MF **1**. The results for the study of solvent influence on the physico-chemical properties of magnetic samples are presented in Table 4.

Water-based MFs **7**–**10** were screened for *in vitro* cytotoxicity on monolayer tumour cell lines HT-1080 (human fibrosarcoma) and MG-22A (mouse hepatoma) and normal mouse fibroblasts (NIH 3T3). The data for *in vitro* anti-tumour activity and toxicity of the water-based MFs have been studied in comparison with the results obtained for biologically active silylalkanolamines[11] and

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Table 6. Magnetic fluid therapeutic indexes (TI_{MF}) for MFs **7**–**10** and **17**[a]

	TI_{MF}			
	HT-1080		MG-22A	
No.	CV	MTT	CV	MTT
7	2.8	18.5	2.5	1.9
8	9.0	19.9	9.1	10.0
9	3.0	4.6	5.3	5.6
10	4.4	22.0	6.0	3.7
17[a][10]	7.0	11.4	12.7	15.3

[a] Contains silylalkanolamine **A1.**

Table 7. K_{OA} and K_A determined for magnetic fluids **7**–**10** and **17**[a]

	HT-1080				MG-22			
	CV		MTT		CV		MTT	
No	K_{OA}[b]	K_A[c]	K_{OA}[b]	K_A[c]	K_{OA}[b]	K_A[c]	K_{OA}[b]	K_A[c]
7	0.7	0.12	4.1	1.1	0.4	0.4	0.3	0.2
8	2.2	6.4	4.4	4.6	1.4	4.1	1.8	3.1
9	0.8	–	1.0	–	0.8	–	1.0	–
10	1.1	0.3	4.9	1.7	1.0	0.15	0.7	0.6
17[a][10]	1.75	14	2.5	22.8	2.0	18.1	2.7	15.3

[a] Contains silylalkanolamine **A1.**
[b] Data of oleic acid cytotoxity[10] have been used for calculation.
[c] Data of A1-A5 cytotoxity[11] have been used for calculation.

oleic acid.[10] The experimental evaluation of cytotoxic properties is presented in Table 5.

All the water-based MFs **7**–**10** are non-toxic and possess high NO-induction ability. The absolute values of these parameters for MFs in almost all cases essentially exceeded the corresponding ones for silylalkanolamines and oleic acid.[10,11] MFs **7, 8** and **10** exhibited a moderate cytotoxic effect concerning human fibrosarcoma HT-1080 (IC_{50} = 24–49 μg/ml, MTT coloration). Less effective in this test was MF **9** containing non-cytotoxic *n*-octyldimethyl(*β*-dimethylaminoethoxy)silane methiodide.[11] The highest cytotoxicity concerning mouse hepatoma MG-22A was observed for MF **10** (IC_{50} = 88 μg/ml, CV coloration) and MF **8** (IC_{50} = 97 μg/ml, MTT coloration). MF **8** possessed the lowest cytotoxicity concerning the normal 3T3 cell line, had high NO-generation activity and was a non-toxic compound (LD_{50} = 4208 mg/kg).

It was expected that the cytotoxicity of MFs **7**–**10** was lower in comparison with silylalkanolamines due to the inclusion of low or non-cytotoxic iron oxide and oleic acid. Since it has been determined that the MFs synthesized (Fe_3O_4:OA : A2–A5) are non-toxic compounds, we calculated the magnetic fluid therapeutic index (TI_{MF}), as the ratio of effective dose for normal cells to its effective dose for tumour cells to reveal the most effective magnetic fluid. This index is similar to the therapeutic index but is characterized by the ratio of toxic dose for normal cells (μg/ml) to toxic dose for tumour cells (μg/ml) instead of the ratio of LD_{50} (mg/kg) to the effective dose (mg/kg). This approach to the interpretation of the results is legitimate due to fact that the LD_{50} was estimated on the basis of the corresponding toxic doses for normal cells. The magnetic fluid indexes (TI_{MF}) are presented in Table 6. Values of TI_{MF} were within 2.8–9.0, the highest index being determined for MF **8** (TI_{MF} = 9.0).

TI_{OA} and TI_A were also calculated using the published data on the cytotoxicity of oleic acid[10] and silylalkanolamines[11] to evaluate the therapeutic efficacy of magnetic fluids in comparison with their components. For this purpose the coefficients K_{OA} and K_A calculated as ratios of TI_{MF} to TI_{OA} and TI_{MF} to TI_A were determined. These coefficients allowed the therapeutic indexes of magnetic fluids to be compared with the therapeutic index of their biologically active components (oleic acid and silylalkanolamine). If the coefficient was ≥1, this could be regarded as a positive fact

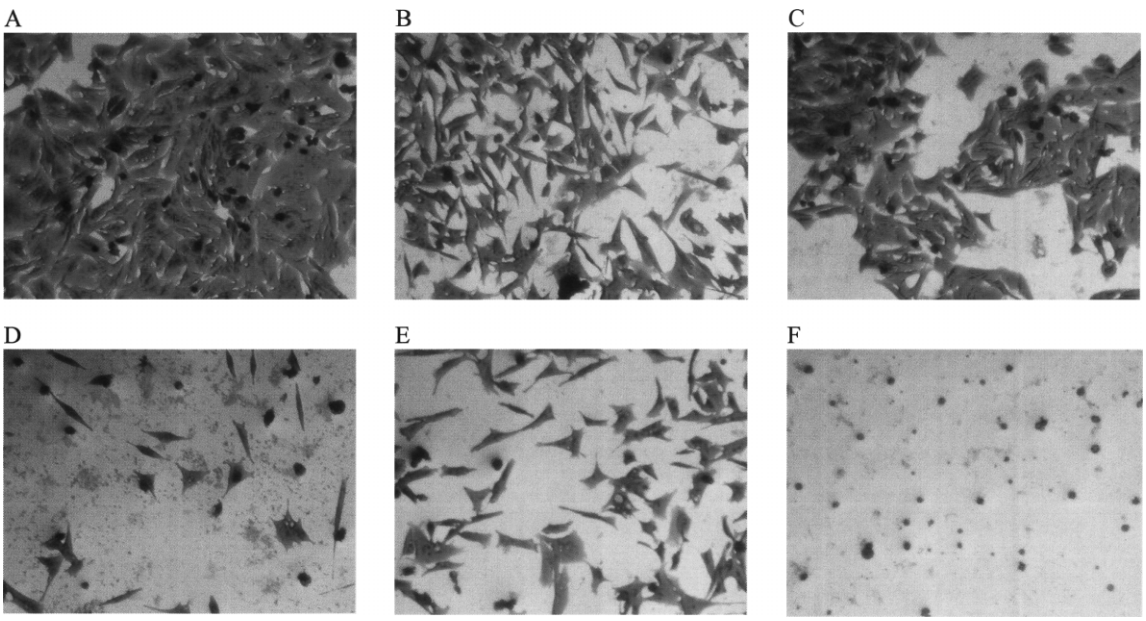

Figure 4. (A) View of mouse hepatoma MG-22A cells (24°, 72 h) visualized with crystal violet (300×); (B) view of mouse hepatoma MG-22A cell phenotype with magnetic fluid **7** in dose 120 μg/ml; (C) **8** (48.75 μg/ml); (D) **9** (500 μg/ml); (E) **10** (87.5 μg/ml); (F) with oleic acid (100 μg/ml).

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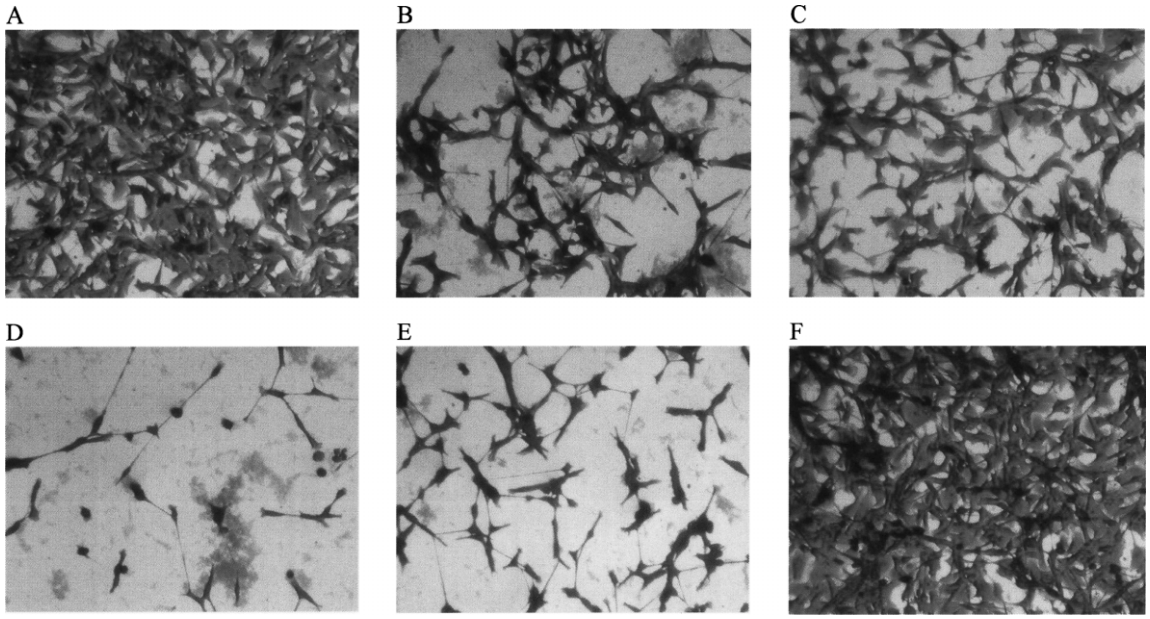

Figure 5. (A) View of human fibrosarcoma HT-1080 cells (24°, 72 h) visualized with crystal violet (300×); (B) view of human fibrosarcoma HT-1080 cell phenotype with magnetic fluid **7** in dose 120 μg/ml; (C) **8** (48.75 μg/ml); (D) **9** (500 μg/ml); (E) **10** (87.5 μg/ml); (F) with oleic acid (10 μg/ml).

in favor of the magnetic fluid. Use of this coefficient is convenient for a series of compounds. The K_{OA} and K_A obtained for MFs **7**–**10** are presented in Table 7.

In most cases K_{OA} and K_A significantly exceed 1. MFs **8** and **17** (contains **A1**)[10] had K_{OA} and $K_A > 1$ in all the tests. This can be regarded as promising result in favor of usage of MFs as therapeutic agents.

The influence of the MFs **7**–**10** on the phenotype of mouse hepatoma MG-22A and fibrosarcoma HT-1080 cells was examined. Figures 4 and 5 show the morphological changes after 72 h, with visualization by crystal violet. Figures 4(A) and 5(A) show the morphological structure of mouse hepatoma and human fibrosarcoma (controls). It was revealed that MFs **7**–**10** have a strong effect on tumour cell morphology. Their dose-dependence and tissue specificity was also observed. Tumour cells have the tendency to form colonies. The cell phenotype changes depending on the MF type, dose and tumour cell line.

Conclusions

The new-water soluble magnetic nanoparticles, bearing absorbed cytotoxic organosilicon choline derivatives accepted as silyl prodrugs, with prolongated action, were synthesized. Water-based magnetic fluids containing corresponding cytotoxic magnetosomes were obtained and their physico-chemical characterization and biological investigation were carried out.

The results presented demonstrate that the procedure for synthesis of model double-coated magnetic nanoparticles containing silylalkanolamines is a promising route for the preparation of magnetosomes functionalized with low molecular biologically active compounds of the same type.

Magnetization of magnetic fluids was obtained within 0.0004–0.0031 emu/g. Concentration of the magnetic nanoparticles was 0.0004–0.0033%. The size of iron oxide core was 7.6–10.2 nm. It was shown by the magnetization data that one water treatment of powdery samples was enough to obtain 68–100% of the maximal possible concentration of magnetite in the corresponding magnetic fluids.

The MFs synthesized possess low or moderate cytotoxic effects concerning human fibrosarcoma and mouse hepatoma tumour cell lines, exhibit high NO-induction ability and have reasonably low acute toxicity (LD_{50} = 3020–4208 mg/kg). *In vitro* experiments have shown biocompatibility of the obtained magnetic fluids. MF **8** was found to be the most effective cytotoxic magnetic fluid among a number of parameters determined. The MFs studied revealed a strong effect on tumour cell morphology. These encouraging results obtained form the basis for further research activity.

Acknowledgments

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