MAGNETITE/OLEIC ACID NANOPARTICLES POSSESSING IMMOBILIZED ANTITUMOUR TETRAHYDROISOQUINOLINE DERIVATIVES

SEGAL[1] I., ZABLOTSKAYA[1] A., MAIOROV[2] M., ZABLOTSKY[2] D., BLUMS[2] E., ABELE[1] E., SHESTAKOVA[1] I.
[1]Latvian Institute of Organic Synthesis, 21 Aizkraukles, Riga, LV-1006, Latvia
[2]Institute of Physics, University of Latvia, 32 Miera, Salaspils LV-2169, Latvia
E-mail: seg@osi.lv

Abstract: Superparamagnetic magnetite/oleic acid nanoparticles bearing lipid like N-(2-hydroxyethyl)-1,2,3,4-tetrahydroisoquinoline derivatives possessing antitumour properties have been synthesized. Methods of magnetogranulometry, DLS measurements and XRD analysis have been employed to investigate their morphology and properties. *In vitro* cell cytotoxicity and itracellular NO generation caused by the water magnetic fluids of obtained nanoparticles were examined concerning tumour HT-1080 and MG-22A and normal NIH 3T3 cell lines.

1. Introduction

The progress in the field of creation and employment of different magnetic nanosystems has a large impact on biomedicine and opens new opportunities for their widespread medical application such as drug delivery [1], MRI contrast enhancement [2], antitumour therapy, which combines hyperthermia [3] and chemotherapy [4] and some others.

Our research team activity deals with targeted searching of medical remedies based on iron oxide nanoparticles, functionalized with wide spectrum of low molecular compounds possessing different kinds of biological activities and potential prolongated action. Recently water based magnetic fluids (MFs), containing model cytotoxic magnetosomes with amphiphilic trialkylsiloxyalkylamines, organosilicon choline and colamine derivatives [5], have been obtained according to the developed original approach. It has been demonstrated that resulting magnetic fluids revealed magnetic properties and affected tumour cell lines [6]. The obtained positive results make it possible to expand the research involving more complex biologically active molecules using the same principle methodology.

2. Presentation of the problem

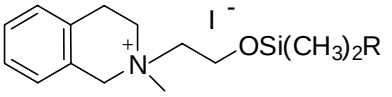
The aim of the present investigation is synthesis, determination of morphology and biological study of magnetic nanostructures, assembling a final product composed of natural components: magnetite, the oleic acid and cytotoxic N-methyl-N-(2-trialkylsiloxyethyl)-1,2,3,4-tetrahydroisoquinolinium iodides anchored to the surface, to evaluate their efficacy as antitumour agents. The tetrahydroisoquinoline ring system is an important structural fragment, which is commonly encountered in naturally occurring alkaloids with interesting biological activities [7]. The parent alkanolamine, N-(2-hydroxyethyl)-1,2,3,4-tetrahydroisoquinoline, was modified by introduction of organosilicon group using decyldimethylsilane or hexadecyldimethylsilane into its side chain and subsequent quaternization of heterocyclic nitrogen atom with methyl iodide [8]. Thus obtained organosilicon amphiphilic heterocyclic choline analogues **1** and **2**, which we further used for the preparation of nanoparticles, can be considered as lipid like molecules: they contain lipophilic tails and are able to interact with the oleic acid shell as the first surfactant, forming cell membrane resembling structure around the magnetite core.

The desired superparamagnetic nanoparticles have been synthesized according to the recently developed synthetic procedure for linear trialkylsiloxyalkyl amines [6]. Thus created structures can provide the interaction of magnetic nanoparticles with cells via nanoparticle

shell and cell membrane fusion. Due to their original unusual magnetosome like structure, containing noncovalently linked silyl prodrugs [9] (the second surfactant) with oleic acid (the first surfactant), complex compositions of this type can be speculated as dual prodrugs; they could be able to ensure the prolongated action of the biologically active compound by the process of gradual desorption, not destroying the whole molecule immediately.
The general steps of synthesis of mixed covered magnetite samples are outlined in Scheme 1.

Fe_3O_4
Magnetite
3

OA: $CH_3(CH_2)_7CH{=}CH(CH_2)_7COOH$
$[THiQ(Me)CH_2CH_2OSiMe_2R]^+I^-$:
1, **6**, **8**, **10**, **12**: R = $C_{10}H_{21}$
2, **7**, **9**, **11**, **13**: R = $C_{16}H_{33}$



Fe_3O_4/OA
Toluene solution
4

Fe_3O_4/OA/1 or **2**
Toluene solutions
6, 7

Fe_3O_4/OA/1 or **2**
Powders
12, 13

Fe_3O_4/OA
Powder
5

Fe_3O_4/OA/1 or **2**
Water solutions
10, 11

Scheme 1. Synthesis of magnetic samples.

Magnetite particles (Fe_3O_4) **3** were obtained by wet synthesis by precipitation from an aqueous solution of Fe(II) sulfate and Fe(III) chloride with excess amount of sodium hydroxide. Initial toluene based MFs **4.1** and **4.2** were synthesized by coating of magnetic particles with OA as surfactant according to the described procedure [10] and differ by their content. The residue, which was left after the fluid **4.1** was decanted, was treated with toluene to give MF **4.2**. For determination of nanoparticles content magnetic powders **5.1** and **5.2** were prepared from initial MFs by treating them with acetone. Magnetic powders **8** and **9** were prepared by shaking of MFs **4.1** and **4.2** with corresponding tetrahydroisoquinoline derivatives and consecutive treating of obtained solutions **6** and **7** with acetone. Magnetic powders **8** and **9** were treated with water to produce water solutions **10** and **11** of nanoparticles containing **1** and **2**, correspondingly. In case of powder **9.3** shaking was replaced by US sonication to produce **11.5**. Water soluble powders **12** and **13** were obtained by solvent evaporation from the corresponding water solutions.
The method of magnetogranulometry has been applied for investigation of magnetic properties and for determination of diameter of iron oxide magnetic core. Magnetization curves were recorded at different stages of the material treatment for the monitoring magnetic material concentration and its condition as well. As a rule, measurements were done at room temperature for fields to 10 KOe. The most expected particle size at the distribution was used as a particle diameter (d). For distribution width estimation we directly use d_{min} and d_{max} at level 0.5 from distribution. Magnetic properties of colloidal solutions were studied without separating of carrier liquid. The data obtained by the method of magnetogranulometry for different powdery samples (**3**, **5** and **12**) are recorded in Table 1.
As expected, magnetite-oleic acid sample **5.2** (Fe_3O_4/OA) with a higher content of magnetite is characterized by higher magnetization value in comparison with the sample **5.1**.

Table 1. Physico-chemical properties of powdery samples **3**, **5** and **12**.

No.	Content mol/mol	Magnetization $\sigma_{10\ kOe}$, emu/g	Superparamagnetic saturation σ_s, emu/g	Magnetite concentration C, %	Magnetite core size d, nm		
					d	d^1_{min}	d^1_{max}
3	Fe_3O_4	55.8	53.8	58.5	12.4	9.9	14.3
5.1	Fe_3O_4/OA 1.95:1	29.9	29.4	32.0	6.4	4.5	8.7
5.2	Fe_3O_4/OA 5.25:1	40.7	42.9	46.6	8.4	5.9	10.8
12.1	Fe_3O_4/OA/**1** 1.95:1.0:0.65	4.84	4.78	5.20	5.2	4.7	5.9

[1] at level 0.5 from distribution density maximum

Dynamic Light Scattering (DLS) technique was used for measuring the size of colloidal particles in carrier liquid. DLS experiments were performed to determine the effective hydrodynamic diameters of the functionalized nanoparticles. Measurements were taken at 20°C. DLS gives the colloidal particles size distribution based on its translational diffusion coefficients in carrier liquid. The addition of the second biologically active surfactant, tetrahydroisoquinoline derivatives **1** or **2**, is not telling much on the obtained micelles size in the corresponding organic solutions, which ranges within 10.7–17.3 nm (Table 2).

Table 2. Size of micelles for patterns in toluene determined by DLS measurements.

Distribution mode, d (nm)		
Fe_3O_4/OA	Fe_3O_4/OA/**1**	Fe_3O_4/OA/**2**
4.1–12.5 **4.2**–17.3	**6.1**–10.7	**7.1**–13.1

However, the size of micelles in water solutions in most cases is considerably bigger (Table 3). In two cases, the samples (**10.2** and **11.5**) were obtained with small micelles, which is apparently associated with a few changes in the method of preparation.

The yield of water soluble powdery samples **12** and **13** with corresponding organosilicon tetrahydroisoquinolines **1** and **2**, obtained from the initial MF **4.1**, which contained nanoparticles with a lower content of magnetite ranges within 11–16%. And just for the samples obtained from liquid **4.2** with a high content of magnetite, the yields were lower 2–4% (Table 4). It was determined by percentage to the amount of mixed covered powders, obtained from toluene solutions.

The magnitude of magnetization and magnetite concentration were calculated using the same parameters obtained for the corresponding water solutions. The calculated value of magnetization 5.08 emu/g of the powdery sample **12.1**, containing **1**, is in good agreement with the experimentally obtained data – 4.84 emu/g (see Table 1).

The compound **2**, used as the second surfactant for the immobilization, have been chosen from the compounds synthesized [8] as possessing selective cytotoxic action against human fibrosarcoma HT-1080 (IC_{50}=17 and 16 μg/ml**,** CV and MTT coloration correspondingly) and mouse hepatoma MG-22A (IC_{50}=13 and 16 μg/ml**,** CV and MTT coloration correspondingly) cell lines and as non-toxic compound (LD_{50}=1083 mg/kg).

Table 3. Size of micelles for patterns in water determined by DLS measurements.

No.	Composition	Molar ratio	d, nm
10.1	Fe_3O_4/OA/**1**	1.95 : 1.0 : 0.65	229
10.2	Fe_3O_4/OA/**1**	1.95 : 1.0 : 1.2	29
11.1	Fe_3O_4/OA/**2**	1.95 : 1.0 : 1.2	260
11.2	Fe_3O_4/OA/**2**	1.95 : 1.0 : -	215
11.3	Fe_3O_4/OA/**2**	1.95 : 1.0 : 0.95	230
11.4	Fe_3O_4/OA/**2**	5.25 : 1.0 : -	225
11.5	Fe_3O_4/OA/**2**	5.25 : 1.0 : -	44

‘-’ – not determined

Table 4. Physico-chemical characterization and yield of water soluble nanoparticles **12**, **13**.

No.	Composition, molar ratio	σ^a, emu/g	C^a, %	d^b, nm	Yield, %
12.1	Fe_3O_4/OA/**1** 1.95:1.0:0.65	5.08	5.0	4.8	11
12.2	Fe_3O_4/OA/**1** 1.95:1.0:1.2	-	-	5.0	-
13.1	Fe_3O_4/OA/**2** 1.95:1.0:1.2	2.78	2.78	5.1	16
13.2	Fe_3O_4/OA/**2** 1.95:1.0:-	-	-	5.5	-
13.3	Fe_3O_4/OA/**2** 1.95:1.0:0.95	9.29	10.63	6.8	13
13.4	Fe_3O_4/OA/**2** 5.25:1:-	-	-	9.2	2
13.5	Fe_3O_4/OA/**2** 5.25:1:-	19.39	18.37	9.6	4

[a]Magnetization $\sigma_{10\ kOe}$ and magnetite concentration *C*, calculated according to the corresponding data for **10** and **11**; [b]Magnetite core size, determined in water solutions **10** and **11**; ‘-’ – not determined

Aqueous MF **11.3** of mixed covered magnetite nanoparticles, containing oleic acid and organosilicon derivative of tetrahydroisoquinoline **2**, was tested for cytotoxicity on monolayer human fibrosarcoma HT-1080 and mouse hepatoma MG-22A tumour cell lines and normal mouse fibroblasts NIH 3T3. It demonstrated high NO-generation ability, revealed selectivity against tumour cell lines and specificity concerning mouse hepatoma MG-22A cells, in contrast to the sample, containing its aliphatic analogue (Table 5).

The sample **11.3** influenced mouse hepatoma MG-22A cell morphology. Visible inclusions were present in cell cytoplasm, which probably accumulate the compound. The amount depends on the cell type and affects the degree of cytotoxic effect.

Table 5. *In vitro* cell cytotoxicity and itracellular NO generation caused by water MF **11.3**.

Compound	HT-1080			MG-22A			NIH 3T3
	IC_{50}[a] CV	IC_{50}[a] MTT	NO[b] CV	IC_{50}[a] CV	IC_{50}[a] MTT	NO[b] CV	IC_{50}[a] NR
11.3 Fe_3O_4/OA/**2** 1.95:1.0:0.95	0.22	0.13	400	0.09	0.08	450	0.32
Fe_3O_4/OA/*[11]	0.11	0.05	800	0.11	0.10	700	0.98

* - $[(C_{16}H_{33})Me_2SiOCH_2CH_2NMe_3]^+I^-$; [a]Concentration (mg/ml) providing 50% cell killing effect (CV, MTT or NR coloration); [b]NO concentration (CV coloration), determined according to the procedure [12]; $r<0.05$

3. Conclusions

Novel water soluble magnetite based nanoparticles precoated with oleic acid and bearing organosilicon heterocyclic choline derivatives have been created. Water magnetic solution of nanoparticles with immobilized cytotoxic organosilicon heterocyclic choline analogue, N-(2-dimethylhexadecylsiloxyethyl)-N-methyl-1,2,3,4-tetrahydroisoquinolinium iodide, exhibited cytotoxic effect on human fibrosarcoma HT-1080 and mouse hepatoma MG-22A cell lines, which surpassed the same against normal cells. Incorporation of the synthesized nanoparticles into cells and their strong effect on MG-22A cell morphology have been observed.

It has been demonstrated that resulting magnetic fluids revealed superparamagnetic properties and affected tumour cell lines. The data are in agreement with our earlier obtained results for aliphatic choline analogues.

4. References

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