Synthesis of glycidoxy spacer on the surface of magnetic nanoparticles and immobilization of albumin

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Abstract. The glycidine spacer was synthesized from three different solvents. It is shown that the samples synthesized from benzene have the highest efficiency. Human albumin was immobilized onto a glycidine spacer. A mechanism for the interaction of albumin with a glycine spacer is proposed. An analysis of the determination of albumin adsorbed by magnetic nanoparticles was performed in this work.

1. Introduction
The reaction of the glycidine group with amines has been known [1] for a long time and is used to crosslink various, mainly polymeric materials [2, 3]. It passes with the disclosure of the cycle (Fig. 1). There are various methods [3] for the synthesis of an amino spacer (Fig. 2). At the same time, any preparation having an amino group can be immobilized on the glycidine spacer, or the corresponding conjugate can be synthesized. Thus, in [4], nanoparticles of a gelatin conjugate and (3-glycidyloxypropyl) trimethoxysilane (GPMS) with a diameter of 200 nm were synthesized. The nanoparticles were modified with S-nitrosothiol. As a result of this, a preparation of efficient delivery of NO for the regulation of vascular cells was obtained, releasing 0.12 mmol/mg of nitric oxide for 7 days. Enzyme-functionalized silicon dioxide nanoparticles were obtained by immobilizing HRP antibodies and alpha-fetoproteins on the surface of silica nanoparticles with the help of linking GPMS [5].

![Figure 1](image1.png)

**Figure 1.** Reaction of glycidine group with amines.

![Figure 2](image2.png)

**Figure 2.** Synthesis of amino-spacer.
Since the blood protein albumin consists mainly of amino acids, a completely logical idea arose to sew them with the help of a glycidine spacer on the surface of a nanoparticle. Also, due to the fact that albumin is a transport protein, it is also, crosslinked on the surface of the MNP, may be able to chemisorb drugs.

2. Materials and methods

2.1. Synthesis of MNPs

The synthesis of the magnetic nanoparticles (MNPs) was carried out as follows [6,7].

2.2. Synthesis of the glycidine spacer

For the synthesis of the glycidine spacer, a (3-glycidyloxypropyl) trimethoxysilane (GPMS) reagent (Sigma Aldrich, 440167) was used. The reaction was carried out in a 50 ml pear-shaped glass flask. 2 grams of lyophilized magnetic nanoparticles (MNPs) were filled with 23.75 ml of solvent and 1.25 ml of GPMS was added. The reaction was carried out in a thermostatic cell at a temperature of 80 °C for 2 hours with stirring on a magnetic stirrer. The immobilization scheme is shown in Figure 3. Benzene, toluene and cyclohexane were used as the solvent. The resulting product was washed five times with cyclohexane, and then freeze-dried at −50 °C and an absolute pressure of 3 Pa in a freeze-dried VaCo 2 (ZirBus, Germany).

![Figure 3. Synthesis scheme of glycidine spacer.](image)

2.3. Determination of the number of spacer by the silicon content

The number of the spacer can be calculated from the silicon content in the synthesis using GPMS, as a chemical substance containing Si in its composition. The analysis was carried out as follows. 50 mg of nanoparticles modified by GPMS were taken and their mineralization was carried out by wet ashing, for which they were boiled in 20 ml of concentrated nitric acid diluted with distilled water in a 1:1 ratio until completely dissolved in a 250 ml flat-bottomed conical flask. The cooled mineralization was adjusted to 100 ml with distilled water. The mineralize thus obtained was analyzed for the content of silicate ion by a spectrophotometric method using molybdenum blue [8]. For this, a 2 ml aliquot sample was placed in a 100 ml volumetric flask, 10 ml of iron chloride II solution at a concentration of 2 g/l and 10 ml of ammonium heptamolybdate solution with a concentration of 0.05 g/ml were added. The pH was set at 1.3 ± 0.1 and left for 15 minutes. Then 5 ml of sulfuric acid was added to neutralize the phosphate complex, 5 ml of reducing agents each - a solution of oxalic acid with a concentration of 0.08 g/ml and a mixture of 2.5 g of citric acid and 0.5 g of ascorbic acid per 100 ml of distilled water. The solution was stirred and left for 15 minutes for color development, adjusted to the mark with distilled water, and the relative optical density was measured on a Unico 2502S spectrophotometer (USA) at a wavelength of 830 nm in cuvettes with a layer thickness of 1 cm.

2.4. Formation of albumin coating

Albumin was immobilized on an LS-220 stirrer at a frequency of 300 min⁻¹ for 2 hours. To this end, 50 mg of MNP with the glycidine spacer were placed in a 15 ml polypropylene tube and 2 ml of a 20% aqueous solution of albumin was added. Human albumin was used (Baxter AG, Vienna, Austria). After sorption, the resulting preparation was washed five times with magnetic separation. Presumably, the reaction proceeds with ring opening and the interaction of the resulting radical with the primary amine of the terminal amino group. The meaning of the interaction can be illustrated by the scheme of interaction of the spacer with a single alpha amino acid tyrosine (Figure 4).
2.5. Determination of the amount of albumin in the coating by thermogravimetry

The amount of spacer and chemisorbed albumin was determined by thermogravimetric method when heated to a temperature of 900 °C in air at a rate of 10 °C/min on a Setaram Setsys Evolution instrument. It was assumed that the whole organic shell consists of a spacer or albumin and burns, interacting with oxygen in the air.

3. Experimental results

3.1. The results of determining the amount of spacer by silicon

The result of determining the amount of spacer by silicon is shown in Table 1. The largest amount of spacer is observed during the synthesis from cyclohexane. The value of the specific surface area of this sample is the lowest. Apparently, this suggests the formation of a thick polymer shell, including agglomerates of nanoparticles. Despite the high formal density of vaccination, one should expect low effectiveness in the immobilization of drugs on this sample. During the synthesis from benzene, a high specific surface area is observed and, relative to the sample from toluene, a high content of the spacer. A high efficacy should be expected in the immobilization of drugs on this sample.

|                  | Specific surface area, m²/g | Silicon content, μg | Concentration of the spacer, mmol/g | Concentration of the spacer per unit surface, μmol/m² |
|------------------|-----------------------------|---------------------|------------------------------------|------------------------------------------------------|
| Fe₃O₄ initial    | 63                          | —                   | —                                  | —                                                    |
| Fe₃O₄-GPMS from benzene | 70                          | 9.714               | 0.35                               | 4.9                                                  |
| Fe₃O₄-GPMS from toluene | 77                          | 4.000               | 0.14                               | 1.8                                                  |
| Fe₃O₄-GPMS from cyclohexane | 42                          | 35.429              | 1.26                               | 30.0                                                 |

3.2. Determination of the amount of albumin in the coating by thermogravimetry

The results of thermogravimetric analysis of samples obtained from benzene are shown in Figure 5 (a). As the thermogram shows, there are two sections. One of them is low-temperature, corresponds to the...
burning out of the organic shell containing a glycine spacer, the second, at a higher temperature, corresponds to the additional oxidation of magnetite to a higher oxide. The results of the analysis of samples with immobilized albumin are shown in Figure 5 (b). As can be seen from the thermogram, with the immobilization of albumin in the low-temperature region of the destruction of the polymer shell, another stage is added. Apparently, it corresponds to protein denaturation - albumin. This corresponds to the literature data [9], where it is stated that all protein denatures at temperatures below 100 °C. Then, there is a two-stage burnout of denaturation products, which can continue up to 450 °C [10]. The average albumin content ranges from 0.5 to 3%.

**Figure 5.** The results of thermogravimetric analysis of samples with a glycidin spacer: (a) synthesis from benzene; (b) synthesis from benzene followed by immobilization of albumin.

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