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Sorbent for the separation of enantiomers of amino acids based on silica gel modified with stabilized Au nanoparticles

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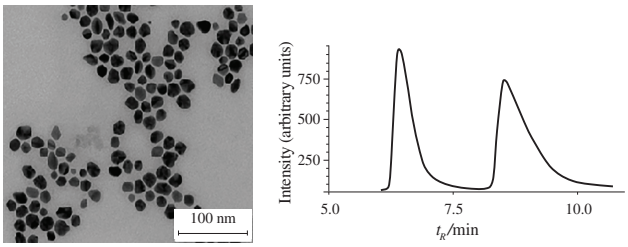
Macrocyclic antibiotic eremomycin was revealed to be capable of stabilizing gold nanoparticles, and these eremomycin-stabilized nanoparticles can be consequently adsorbed on the sorbent surface at the selector content of 0.017 mmol g^{-1}. Silica gel modified with the adsorbed nanoparticles and eremomycin demonstrated a high enantioselectivity towards amino acids. The best separation of enantiomers was achieved in the cases of tryptophan, 3,4-dihydroxyphenylalanine and phenylalanine, the analysis time being less than 10 min.



Over the last decade, various organic nitrogen-containing compounds (*e.g.*, amines, polymers, and proteins) have been found to serve as reducing agents for $AuCl_4^-$ and stabilizers of the formed gold nanoparticles (GNPs).[1,2] Such GNPs were less explored than citrate-stabilized GNPs, although the application of amino acids, peptides and proteins as the reducing agents for the GNP synthesis attracted a considerable attention as the 'green' approach, since GNPs functionalized with biomolecules can be used in biosensors and biomedical devices.[3,4]

Some biomolecules possess enantioselective properties, so it is important from both the theoretical and practical aspects to explore the GNP ability to identify enantiomers. Recently, a new spectrophotometric method was suggested for the recognition of glutamine enantiomers in the form of nanoparticles.[5] However, more fruitful results can be achieved in the case of sorbents modified with GNPs stabilized by various chiral selectors.[6–8] Macrocyclic antibiotics are the universal chiral selectors, but their complexes with GNPs remain insufficiently explored limiting mainly by GNPs stabilized with vancomycin. Those nanoparticles have demonstrated a high antibacterial activity against the gram positive bacteria.[9,10] The nanotechnological approach to a drug delivery of antibiotics during the treatment is promising for overcoming an antibiotic resistance in order to reduce their doses. Vancomycin bounded chemically to GNPs stabilized with citrate ions and immobilized on the surface of silica gel has already been employed as a new chiral stationary phase for the enantioseparation of a number of β-blockers (pindolol, metoprolol, oxprenolol, *etc.*).[11]

Eremomycin is another macrocyclic antibiotic, structurally similar to vancomycin. It has been successfully applied to the separation of enantiomers in both HPLC and capillary electrophoresis.[12–17] However, eremomycin-stabilized GNPs have practically been not investigated. There is only one report[18] on the possibility of separating (*RR*,*SS*) enantiomers of (1*RS*)-2-{[(1*RS*)-2-(4-hydroxyphenyl)-1-methylethyl]amino}-1-(3,5-dihydroxyphenyl)ethanol hydrobromide on silica gel, where the antibiotic was linked to a residue of mercaptopropionic acid immobilized on silica gel *via* Au atoms due to the formation of coordination bond with S atoms.

This work was aimed at the obtaining of eremomycin-stabilized GNPs and the sorbent based on silica gel modified *via* their adsorption. To evaluate possible applications of this new sorbent, its hydrophilic, acid-base and enantioselective properties were estimated.

Nanoparticles were synthesized by the prolonged heating of a mixture of $HAuCl_4$ (100 μg ml^{-1}) and eremomycin (1 mg ml^{-1}) in a 0.05 M borate buffer solution. The GNP formation was confirmed by the appearance of a ruby-colored solution. The mean size of eremomycin-stabilized GNPs was 14.2 nm according to the transmission electron microscopy (TEM) data (Figures 1 and S1 in the Online Supplementary Materials). The prepared GNPs were immobilized on the surface of silica gel *via* their physical adsorption saturating the surface of silica gel. After the modification of silica gel with eremomycin-stabilized GNPs, the resulting sorbent was examined by scanning electron microscopy (SEM) (Figures 2 and S2) and diffuse reflection (DR) methods. It was shown that the GNPs have been almost uniformly adsorbed on the surface of spherical silica gel particles, while the Au content on the surface was 1.32 wt% according to the microanalysis (EPMA). The surfacial content of eremomycin,

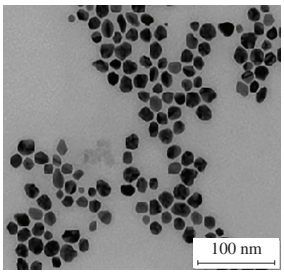


Figure 1 TEM image of the obtained gold nanoparticles.

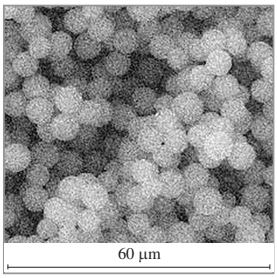


Figure 2 SEM microphotograph of the Kromasil–GNP–eremomycin sorbent.

calculated from the chlorine content, was 0.017 mmol g^{-1}, which is significantly lower than that during a chemical fixation of eremomycin on epoxy silica gel (0.07 mmol g^{-1}).[14] The absorption maximum in the region of 540 nm in the DR spectrum proves the presence of GNPs in a non-aggregated state on the sorbent surface (Figure S3). Parameters of the synthesized sorbent surface, as well as unmodified silica gel, were estimated by the low-temperature nitrogen adsorption method (Figure S4). Sorbent containing the GNPs possesses a smaller surface area (260 m^2 g^{-1}) than that of unmodified silica gel (320 m^2 g^{-1}), which can be explained by partial closure of the silica gel pores by the eremomycin-stabilized GNPs as evidenced by the decrease in the total pore volume of the modified sorbents by 17%.

The hydrophilic-hydrophobic and acid-base properties of the sorbent were investigated using mixtures of uridine, 5-methyluridine, 2-deoxyuridine, caffeine, theophylline, and theobromine. These substances and their mixtures were selected due to the data reported previously.[18] Properties of silica gels modified chemically with eremomycin and those modified by the adsorption of GNPs with eremomycin were compared.

The degree of hydrophobic interactions between the stationary phase and test compounds was calculated from the methylene (hydrophobic) selectivity $\alpha(CH_2)$, which can be obtained by comparing the retention factors (k) for 5-methyluridine and uridine. Since 5-methyluridine is more hydrophobic than uridine, it would be eluted before uridine in the case of retention affected by the hydrophobic interactions. The hydrophilic interactions were evaluated using a selectivity towards hydroxyl group. This selectivity factor α(OH) can be derived from the comparison of k values for uridine and 2-deoxyuridine. The greater selectivity factor α(OH), the more pronounced hydrophilic properties of the stationary phase. To determine the acid-base nature of sorbents, mixtures containing caffeine and isomers of theobromine and theophylline possessing the different acidity constants were employed. The ratio of retention factors of these compounds was calculated (*i.e.*, k_{caf}/k_{theoph} and k_{caf}/k_{theobr}). The resulting selectivity factor lower than 1 means that the phase is basic; if it is equal to 1, the phase is neutral; and the phase is acidic if it is higher than 1.[18] Toluene was used as the non-retainable component. As one can see from the calculated parameters (Table 1), the column filled with silica gel modified with eremomycin and GNPs demonstrates both the hydrophilic and weak hydrophobic properties.

Table 1 The methylene (hydrophobic) selectivity factor $\alpha(CH_2)$, the selectivity towards OH group α(OH), and the retention factors k_{caf}/k_{theoph} and k_{caf}/k_{theobr} for the obtained sorbents.[a]

Sorbent	$\alpha(CH_2)$	α(OH)	k_{caf}/k_{theobr}	k_{caf}/k_{theoph}
Kromasil–GNPs–eremomycin	0.88	1.21	0.89	0.70
Kromasil–eremomycin	0.87	1.50	0.49	0.40

[a] Mobile phase was MeCN : ammonium acetate buffer (90 : 10), 20 mM, pH 4.7. The flow rate of mobile phase was 0.5 ml min^{-1}.

Table 2 Influence of the mobile phase composition on the chromatographic parameters of the used amino acids.[a]

Mobile phase	Substance	k_1	k_2	α	R_s
MeCN–H_2O (20 : 80)	Tryptophan	0.82	1.44	1.76	2.62
	Phenylalanine	0.54	1.10	2.04	3.15
	4-Chlorophenylalanine	0.53	0.84	1.58	2.45
	Alanine	0.40	0.55	1.38	1.05
	Methionine	0.48	0.55	1.15	0.48
	DOPA	0.59	1.41	2.39	2.39
MeOH–H_2O (20 : 80)	Tryptophan	2.21	3.59	1.62	2.06
	Phenylalanine	2.02	3.24	1.60	1.87
	4-Chlorophenylalanine	1.63	2.48	1.52	2.26
MeCN–H_2O (10 : 90)	Tryptophan	5.05	7.81	1.34	1.55
	Phenylalanine	5.92	7.94	1.34	1.34
MeCN–H_2O (80 : 20)	Tryptophan	12.27	16.41	1.28	1.47
	Phenylalanine	17.42	22.33	1.28	1.22

[a] k_1 and k_2 are retention factors of the first and second enantiomer, respectively; α is the selectivity coefficient; and R_s is the resolution of enantiomers.

The stationary phases exhibit basic properties. The presence of GNPs on the surface reduces the hydrophilicity and basicity of column, which can be explained by the linking of part of the eremomycin amino groups to the Au atoms.

The separation of substances on such sorbents was carried out either in the polar-organic mode using mobile phases with a high content of organic solvent (usually MeCN) with polar additives (AcOH, Et_3N, H_2O) or in the reversed-phase mode. According to other works,[11,15,19] silica gels modified with eremomycin possess a good enantioselectivity with respect to amino acids and profens. Thus, the enantioselective properties of considered sorbent were estimated using tryptophan, phenylalanine, 4-chlorophenylalanine, alanine, methionine, serine, aspartic acid, and 3,4-dihydroxyphenylalanine (DOPA). The eremomycin enantioselectivity towards them was the highest.

The evaluation was started in the reversed-phase mode, eluting the substances with a mixture of MeCN and H_2O in the ratio of 20 : 80 (v/v). It was possible to separate the enantiomers of almost all the studied amino acids except for serine and aspartic acid, which were eluted from the column in blurred zones. A considerable retention of these amino acids may be attributed to the presence of additional OH and COOH groups, respectively, in their molecules, which can interact with Au atoms. The acquired results are given in Table 2. The chromatogram of racemic mixture of 4-chlorophenylalanine was also recorded (Figure S5). Then, the nature and content of organic solvent in the mobile phase were varied. A replacement of MeCN with MeOH increases the retention of amino acids but decreases the selectivity and resolution of peaks of the enantiomers. The increase in H_2O content increases the retention of amino acids, especially phenylalanine and 4-chlorophenylalanine but hampers the separation of enantiomers due to the strong peak broadening.

A good separation of the enantiomers of tryptophan, phenylalanine and 4-chlorophenylalanine was achieved in the

Table 3 Comparison between the enantioselectivities (α) of experimental and Nautilus E columns for the studied amino acids.[a]

Column	Tryptophan	Alanine	Phenylalanine	DOPA
Experimental	1.76	1.38	2.04	2.39
Nautilus E	2.35	2.86	4.33	2.65

[a] The experimental column was 15 cm long, and the selector content was 0.017 mmol g^{-1}. Nautilus E column was 25 cm long, and the selector content was 0.072 mmol g^{-1}.

case of high MeCN content in the mobile phase (80–90 vol%), however the separation time was long and exceeded 50 min in some cases. The resulting sorbent worked stably for four months and only after that, the retention and selectivity for the separation of enantiomers decreased, probably due to some partial desorption of eremomycin from the surface of silica gel.

We compared enantioselectivities of the experimental column and commercial Nautilus E column with eremomycin. As one can see from Table 3, the enantioselectivity of Nautilus E column is higher, which is consistent with the greater density of eremomycin on the sorbent surface.[15] However, on silica gel modified with GNPs, the enantiomers of amino acids were successfully separated.

In conclusion, we have designed, prepared and practically evaluated the new sorbent based on modified silica gel for the column chromatography. The main advantage of this sorbent is its simple synthesis and the lower consumption of selector as compared to a commercial one.

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Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.1016/j.mencom.2019.11.035.

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