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1 **Mesoporous silica adsorbents modified with amino polycarboxylate**
2 **ligands – functional characteristics, health and environmental**
3 **effects**

4 Gulaim A. Seisenbaeva,^{a*} Lamiaa M.A. Ali,^{b,c,d,‡} Ani Vardanyan,^{a,‡} Magali Gary-
5 Bobo,^d Tetyana M. Budnyak,^{e,f} Vadim G. Kessler,^a Jean-Olivier Durand^{b*}

6 a - Department of Molecular Sciences, BioCenter, Swedish University of Agricultural Sciences, Box
7 7015, SE-75007, Uppsala, Sweden.

8 b - ICGM, Univ. Montpellier, CNRS, ENSCM, Case 1701, Place Eugène Bataillon, CEDEX 05, 34095
9 Montpellier (France)

10 c - Department of Biochemistry, Medical Research Institute, University of Alexandria, 21561
11 Alexandria, Egypt.

12 d IBMM, Univ Montpellier, CNRS, ENSCM, Montpellier. 15 Avenue Charles 9 Flahault, 34093
13 Montpellier Cedex 05 (France)

14 e - Department of Materials and Environmental Chemistry, Stockholm University, Svante Arrhenius
15 väg 16 C, 106 91 Stockholm, Sweden

16 f - Chuiko Institute of Surface Chemistry of National Academy of Sciences of Ukraine, 17 General
17 Naumov Str., 03164 Kyiv, Ukraine.

18 **KEYWORDS:** Kromasil® silica, hybrid adsorbent, REE extraction and separation,
19 amino carboxylate ligands, health and environmental effects.

20 Corresponding authors:

21 Gulaim A. Seisenbaeva

22 **Email:** gulaim.seisenbaeva@slu.se

23 **Postal Address:** Department of Molecular Sciences, BioCenter, Swedish University
24 of Agricultural Sciences, Almas allé 5, Box 7015, SE-75007, Uppsala, Sweden

25 Jean-Olivier Durand

26 **Email:** durand@univ-montp2.fr

27 **Postal Address:** Institut Charles Gerhardt de Montpellier, UMR5253, Université de Montpellier,
28 Place EugèneBataillon, CC 1700 – Bâtiment 17, 34095 Montpellier Cedex 5, France.

29

30 **ABSTRACT:** A series of hybrid adsorbents were produced by surface modification
31 with amino polycarboxylate ligands of industrially available microparticles (MP) of
32 Kromasil® mesoporous nanostructured silica beads, bearing grafted amino propyl
33 ligands. Produced materials, bearing covalently bonded functions as EDTA and
34 TTHA, original Kromasil®, bearing amino propyl ligands, and bare particles, obtained
35 by thermal treatment of Kromasil® in air, were characterized by SEM-EDS, AFM,
36 FTIR, TGA and gas sorption techniques. Adsorption kinetics and capacity of surface-
37 modified particles to adsorb Rare Earth Elements (REE), crucial for extraction in
38 recycling processes, were evaluated under dynamic conditions, revealing specificity
39 matching the ligand nature and the size of REE cations. A detailed comparison with
40 earlier reported adsorbents for REE extraction was presented. The cytotoxicity was
41 assessed using four different types of healthy cells, human skeletal muscles derived
42 cells (SKMDC), fibroblast cells, macrophage cells (RAW264.7), and human umbilical
43 vein endothelial cells (HUVECs), indicating lower toxicity of ligand-free MP than MP
44 bearing amino poly-carboxylate functions. Internalization of the MP inside the cells
45 and release of nitric oxide were observed. In addition, zebrafish embryos were
46 exposed to high concentrations of MP and did not show any pronounced toxicity.

47 **1. INTRODUCTION**

48 Rare Earth Elements (REE) belong to the critical elements for development of
49 modern industries. Their applications in such domains as energy production and
50 storage, both as components in permanent magnets in wind power turbines¹ and as
51 components in NiMH batteries² and as magnetic core materials for electric engines
52 of electrically driven vehicles,³ urges development of new sources of these elements.
53 Both primary mining and recycling of REE require development of powerful
54 extraction and separation techniques that need to be sustainable, not involving large
55 amounts of hazardous reagents and solvents. As an alternative to traditionally used
56 liquid extraction, the application of solid adsorbents has been proposed.⁴ The
57 concept of molecular recognition, earlier applied in solid adsorbents for selective
58 removal of alkali and alkaline earth elements and exploiting crown ether ligands, has
59 been proposed.⁵ Related poly-oxygen donor ligands have been recently used on
60 mesoporous silica matrices, showing appreciable selectivity between different
61 members of the REE series.⁶ As possible other types of ligands can be considered,
62 for example, calixarenes. Adsorbents with calixarenes grafted on silica have been
63 reported for removal of group I and II cation from polluted waters.⁷ Free calixarenes
64 have been used in the past as complexing ligands in solvent extraction of REE.⁸
65 Surface-complexed REE bound to silica matrix *via* calixarene functions have recently
66 been reported as luminescent materials.⁹ Other possible candidates for grafting on
67 silica as ligands for REE extraction are poly N-donor functions discussed in a recent
68 review.¹⁰ These materials rely on multi-step synthesis, involving expensive ligands.

69 An attractive alternative in the choice of ligands for REE extraction and separation is
70 offered by use of amino polycarboxylic acids (APCA), often referred to as
71 complexones, which are large-scale industrial products. They have been broadly

72 recognized for their ability to form highly stable complexes with a broad variety of
73 metal cations ¹¹, and also for their active exploitation in the techniques of
74 complexometric titration ¹² and chelato-therapy ¹³, where ethylene diamine tetra-
75 acetic acid (EDTA) received the main attention. In connection with the use of REE
76 cations in magnetic resonance imaging (MRI) as contrast agents,¹⁴ the interest has
77 been attracted even to larger APCA ligands in REE complexes, such as diethylene
78 triamine penta-acetic acid (DTPA) and Triethylene tetramine hexa-acetic acid
79 (TTHA).¹⁵⁻¹⁸ Later, the relatively facile grafting of these ligands on different polymers
80 ¹⁹⁻²¹ and silica ²² colloid carriers, especially magnetic ones, made the derived hybrid
81 materials attractive as adsorbents for advanced water purification and, more
82 recently, for extraction and separation of REE in hydrometallurgy.²³⁻²⁶ The grafting of
83 APCA ligands occurred *via* formation of an amide bond, originating from
84 condensation of one of the carboxylic functions (Scheme 1). The capacity and
85 selectivity of the produced hybrid adsorbent were depending on the nature of ligand.
86 Ligands with higher number of functional groups demonstrated more pronounced
87 capacity towards larger (lighter) REE cations.^{24,27,28} Recently, the attention was
88 attracted to produce derivatives of mesoporous silica microparticles (MP) in the view
89 of their large surface area and large size, which permitted their use in
90 chromatographic separation applications. Additionally, their separation became
91 easier when magnetic components were involved.²⁹

92 Silica has been listed as “generally recognized as safe” by the FDA.³⁰ For that
93 reason, silica-based nano- or micro-particles became an attractive tool for industrial,
94 environmental and medical applications. The toxicological behavior of nano- or
95 micro-particles was not limited only to the chemical composition but also extended to
96 their sizes, surface charges and shapes.³¹⁻³⁶ Therefore, considerable attention
97 should be paid to studying the impact of silica-based particles on health and
98 environment.³³⁻³⁴

99 In the present paper, we have further developed the surface modification
100 approaches recently reported for industrially available mesoporous silica Kromasil®
101 MP, carrying amino propyl functions,²⁹ and produced adsorbents modified with EDTA
102 and TTHA ligands, awaiting potentially extended difference in capacity and
103 selectivity between adsorbents bearing different ligands. The rationale of this study
104 was based on well-demonstrated difference in capacity and selectivity of dense
105 nanoadsorbents modified with ligands derived from EDTA, DPTA and TTHA. While
106 they contained binding functions of the same kind, in the first hand, carboxylic
107 groups, and tertiary amines, their mode of interaction with REE differed very
108 significantly, because of different organization on the surface. Increase in the ligand
109 size was observed to cause differences in the mode of surface binding for REE,
110 resulting in pronounced selectivity towards distinct REE in the series, especially, on
111 release.²⁷ In the present case, a new mode of surface ordering could be expected for
112 mesoporous structures of Kromasil® derived materials. Morphological, structural and
113 functional characteristics of the new materials were determined, revealing much

114 more complex adsorption mechanisms as compared to previously expected.
115 Toxicological behavior of new materials was investigated on four healthy cell lines.
116 They were selected with respect to the most probable ways of exposure and
117 retention in the body. These particles, expected to be used in recycling of REE
118 materials or in water purification, would potentially be able to escape into
119 wastewaters with most probable exposure scenario being the uptake via
120 gastrointestinal tract. The cell lines were thus represented by macrophage cells as
121 the most common cells of the immune system, fibroblast cells as the most common
122 cells of the connective tissue, HUVECs as model for blood vessel cells, and by
123 skeletal muscle-derived cells to evaluate the potential retention effects.

124 In addition, cellular uptake and nitric oxide release were described. Moreover, *in vivo*
125 toxicity study was conducted using zebrafish embryos as a predictive model for
126 assessing the nano- and micro-material toxicity.³⁷ Obtained results were compared
127 to those obtained using original Kromasil® and Kromasil-derived mesoporous silica
128 deprived from organic ligands.

129 **2. MATERIALS AND METHODS**

130 2.1 Chemicals and agents

131 All chemicals used in the experiments were purchased as analytical grade reagents
132 and used as received without further purification. All solutions were prepared in
133 deionized water obtained from a Millipore Direct-Q3 water purification system
134 (France). Ethylene diamine tetra-acetic acid (EDTA, anhydrous, CAS No. 60-00-4)
135 and Triethylene tetramin hexa-acetic acid (TTHA, CAS No. 869-52-3) were
136 purchased from Sigma Aldrich, as well as applied organic solvents, toluene and
137 ethanol. Mesoporous Kromasil® particles (average size 10 µm, average pore size
138 100 Å, APTES-functionalized) were donated by AkzoNobel Special Chemicals AB,
139 Sweden. Hydrated nitrates of REE, Lanthanum(III) nitrate hexahydrate,
140 $\text{La}(\text{NO}_3)_3 \cdot 6\text{H}_2\text{O}$ (CAS No. 10277-43-7), Neodymium(III) nitrate hexahydrate,
141 $\text{Nd}(\text{NO}_3)_3 \cdot 6\text{H}_2\text{O}$ (CAS No. 16454-60-7) and Dysprosium) nitrate hydrate,
142 $\text{Dy}(\text{NO}_3)_3 \cdot x\text{H}_2\text{O}$ (CAS No. 100641-13-2) were purchased also from Sigma Aldrich.
143 Solutions for studies of REE uptake (0.05 M) were produced by dissolving desired
144 masses of solid nitrate salts in MilliQ water. The concentrations of REE solutions
145 were determined by complexometric titration with EDTA using Xylenol Orange as
146 indicator.

147 2.2 Functionalization

148 500 mg of Kromasil® (APTES-bearing) MP and 20 mL dry toluene were placed in a
149 reaction flask and the mixture was put into an ultrasonic bath. To the produced
150 dispersion, 100 mg of dry APCA (EDTA or TTHA) was added. The obtained solution
151 was kept overnight under inert atmosphere at 70 °C with continuous stirring. After 24
152 h, the particles were separated by centrifugation (8700 rpm; 10 min), then were
153 washed 2 times with 5 mL toluene and put into the ultrasonic bath and then

154 centrifuged again (8700 rpm; 10 min). Afterwards, the material was washed with 5
155 mL ethanol, sonicated in ultrasonic bath and was separated by centrifugation (8700
156 rpm; 10 min). The obtained particles were dried under nitrogen atmosphere. A
157 portion of non-functionalized Kromasil® was heat treated at 500°C in air for 2 h to
158 produce purely inorganic material for reference.

159 2.3 Uptake studies

160 Total uptake. 20 mg of particles were placed in a Falcon tube with 20 mL 0.010 M
161 salt of REE and put in a shaker for 21 h. The material was then separated by
162 centrifugation and was washed 3 times with 2.5 mL of MilliQ water. The supernatant
163 and washing waters were united and analyzed by titration, while the particles were
164 analyzed by Scanning Electron Microscopy / Energy Dispersive X-Ray Spectroscopy
165 (SEM-EDS) for control estimation of adsorbed REE content.

166 *Isotherm measurements.* Stock solution of $\text{Ln}(\text{NO}_3)_3$, where Ln = La, Nd, Dy, with 0.2
167 M concentration was diluted for isotherm experiments. Final metal concentration was
168 varying between 0 to 20 mM (0.5 mM, 1 mM, 5 mM, 10 mM and 20 mM). 10 mg of
169 Kromasil-NP samples were mixed with 10 mL of metal solution in a 50 mL plastic
170 tube and put on a shaker for 24 hours. After 24 hours the samples were centrifuged
171 (7000 rpm) and the remaining metal concentration was determined by titration as
172 described above. For each sample, the titrations were repeated 3 times and the
173 average was calculated.

174 *Kinetic experiments.* 30 mg Kromasil-NP were mixed in 30 mL metal solution with
175 initial 10 mM metal concentration. After set interval of times NPs were centrifuged
176 (7000 rpm) for 10 min and 1 mL aliquot was separated to determine the metal
177 concentration in the remaining solution. The sample was first diluted 10 times, and
178 titrated afterwards.

179 2.4 Characterization

180 Particles were morphologically characterized using a SEM-EDS Hitachi TM1000- μ -
181 DeX and Flex-SEM 1000 environmental scanning electron microscopes and a
182 Bruker FastScan Bio atomic force microscope (AFM). Grafting of ligands was
183 monitored with infrared spectroscopy (IR) using a Perkin-Elmer Spectrum 100 and
184 with thermogravimetric analysis (TGA) using a Perkin-Elmer Pyris-1 instrument.
185 Chemical composition was obtained by EDAX analysis using Hitachi Flex-SEM 1000
186 instrument and by elemental microanalysis performed by certified EuroFINs
187 laboratory in Lidköping, Sweden. Specific surface areas and pore size distribution
188 were determined from the low temperature nitrogen adsorption and desorption data.
189 For this process, an automatic sorption analyzer ASAP 2020 (Micromeritics, USA)
190 was used. The samples were degassed at 120 °C during 10 h before measurements.
191 The analysis report was generated automatically using Micromeritics software (for
192 details, please, see the Supplementary materials Tab. TS1 and text). Powder X-ray
193 diffraction patterns of the silica nanoparticles and the Kromasil® MP were recorded

194 using a multifunctional Bruker D8 SMART Apex-II diffractometer operating with Mo-
195 K_{α} radiation.

196 2.5 Cytotoxicity studies

197 *Cell cultures.* Four different healthy cell lines were used; human skeletal muscle-
198 derived cells (SKMDCs), fibroblast cells, murine macrophage cells (RAW264.7), and
199 human umbilical vein endothelial cells (HUVECs).

200 SKMDCs were maintained in F-10 nutrient medium supplemented with 25% fetal
201 bovine serum (FBS), 1% penicillin/streptomycin (P/S), 0.1% insulin, 0.01% fibroblast
202 growth factor (FGF), and 0.01% epidermal growth factor (EGF). Fibroblast cells were
203 maintained in RPMI medium supplemented with 10% FBS and 1% P/S. RAW264.7
204 cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM)
205 supplemented with 10% FBS and 1% P/S. HUVECs were maintained in Endothelial
206 cell Growth Medium 2 supplemented with FBS (2%), EGF (5 ng mL⁻¹), basic FGF (10
207 ng mL⁻¹), insulin-like growth factor (ILGF) (20 ng mL⁻¹), vascular endothelial growth
208 factor (VEGF) (0.5 ng mL⁻¹), ascorbic acid (1 μ g mL⁻¹), heparin (22.5 μ g mL⁻¹),
209 hydrocortisone (0.2 μ g mL⁻¹), 1% P/S.

210 All cell lines were allowed to grow in humidified atmosphere at 37°C under 5% CO₂.

211 *Cell viability assay.* For cell viability experiments, cells were seeded in 96-well plate
212 in 200 μ L of their respective culture medium, 24 h after cell growth; cells were
213 treated with different concentrations of MP and were incubated for 1, 2 and 3 days.
214 Cells treated with the vehicle were considered as control. At the end of the
215 incubation time, cells were incubated with 0.5 mg mL⁻¹ of MTT (3-(4,5-
216 dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide). Three hours after,
217 MTT/medium solution was removed and the precipitated crystals were dissolved in
218 ethanol/DMSO (1:1) solution with shaking for 20 min. The absorbance was
219 measured at 540 nm. The optical density (OD) values were directly correlated with
220 the number of living cells in well. The percentage of living cells (%) was calculated
221 according to the following equation: OD of treated cells/ OD of control x 100

222 The dose-response curves were plotted as the log microparticle concentration in μ g
223 mL⁻¹ versus the percentage of viable cells using Graph Pad Prism 5.0 software (San
224 Diego, CA, USA). Data of control cells are not shown in figures as Log zero is
225 undefined. The mean lethal concentration (LC50) was determined at 50% of cell
226 death.

227 *Nitric oxide (NO) measurement.* RAW264.7 cells were seeded in 96-well plate in 200
228 μ L of their respective culture medium, 24 h after cell growth; cells were treated with
229 different concentrations of MP and were incubated for 1 day. Cells treated with the
230 vehicle were considered as negative control. For positive control, cells were treated
231 with 100 ng mL⁻¹ of lipopolysaccharide (LPS). Nitric oxide release was measured
232 using the Griess reagent (1% sulfanilamide, 0.1% N-[1naphthyl]-ethylenediamine

233 dihydrochloride, 5% phosphoric acid). After the incubation time, culture medium was
234 collected and mixed with equal volume of Griess reagent and incubated for 15 min at
235 room temperature in the dark. Sodium nitrite in cell culture medium was used to
236 generate a standard curve (0 – 100 μM). Absorbance was measured at 540 nm. The
237 concentration was calculated from the derived nitrite standard curve equation.
238 Statistical analysis was carried out using unpaired t-test.

239 *Cellular Uptake.* Fibroblast cells were seeded in fluoroDish™, glass thickness 0.17
240 mm, in 1 mL of their respective culture medium. Twenty four hours after seeding,
241 cells were treated with or without 25 $\mu\text{g mL}^{-1}$ of MP and were incubated for 24 h.
242 Cells were washed three times with their culture medium then exposed to Z-stack
243 imaging using Zeiss LSM780 confocal fluorescence microscopy, 10x objective.
244 Several images were taken along the Z-direction to know whether MP were present
245 inside the cell or not. Images were treated with imageJ program.

246 *In vivo toxicity in zebrafish embryos.* Wild-type AB zebrafish strain was purchased
247 from Zebrafish International Resource Center (ZIRC) as embryos and were raised to
248 adulthood in circulating aquarium system inside environmentally controlled room (28
249 °C, 80% humidity, 14 h light/10 h dark cycle), in the lab's facilities of Molecular
250 mechanisms in neurodegenerative dementia (MMDN), Inserm U1198, Montpellier
251 University, Montpellier.

252 Fertilized embryos were collected and maintained at 28 °C. At 7 hours post
253 fertilization (hpf), embryos were examined under the microscope, and only embryos
254 that developed normally and reached gastrula stage were selected for the study. The
255 7 hpf embryos were placed in 12 well plate (20 embryos per well) and exposed to 4
256 mL water containing 0 or 125 or 500 mg L^{-1} of MP, concentrations were chosen
257 according to previous toxicity studies.³⁸⁻⁴¹ The exposure to the MP was started at 7
258 hpf and ended at 96 hpf. The percentages of survival, mortality and hatching
259 embryos were recorded using Loupe Olympus MVX10 stereomicroscope at 24, 48,
260 51, 56, 72 and 96 hpf.

261 Experiments with zebrafish embryos until 96 hpf are considered as *in vitro* studies
262 according to the EU Directive 2010/63/EU on the protection of animals used for
263 scientific purposes.⁴²

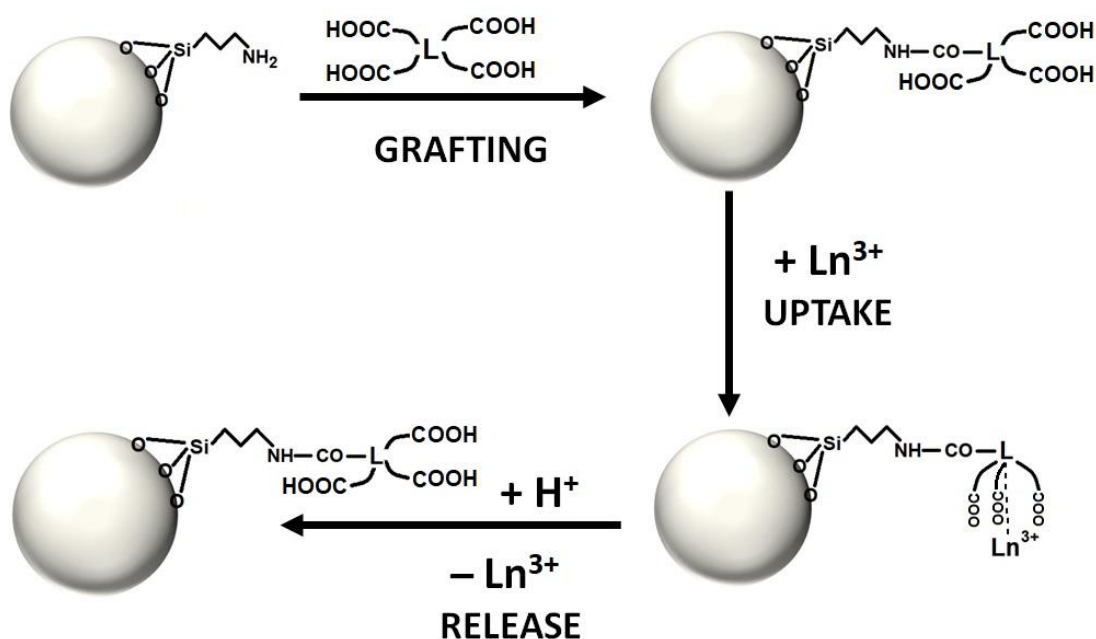
264 **3. RESULTS AND DISCUSSION**

265 In the present study, we aimed at characterization of Kromasil based materials
266 bearing for this system earlier not investigated functions, EDTA and TTHA, and
267 produced their comparison with carboxylate function-free Kromasil® material.

268 **3.1 Functional characteristics of materials**

269 In agreement with the earlier observations on modification of Kromasil® with DTPA,
270 the appearance of the material did not change, provided that the temperature in the
271 course of synthesis remained low enough to avoid polymerization of the APCA

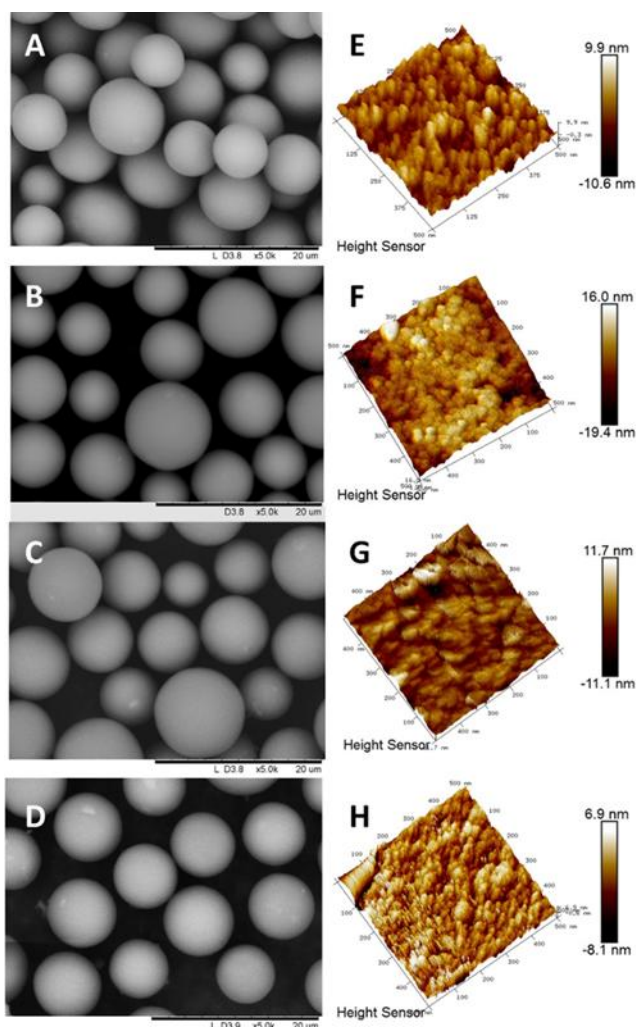
272 ligands (below 80°C as excessive heating results in brownish tone and strongly
 273 decreased capacity in adsorption of metal cations). The attachment of ligands
 274 occurred apparently *via* formation of an amide bond, consuming one carboxylate
 275 group per ligand moiety, as proved below by spectroscopic data (for description of
 276 underlying transformations, please, see Scheme 1). The amide bonds in grafted
 277 ligands were known to possess outstanding chemical stability. Thus for the earlier
 278 investigated DTPA-modified Kromasil the adsorption capacity was investigated in 50
 279 consecutive cycles of uptake and release using 0.1 M nitric acid and showed a
 280 decrease of less than 1%, i.e. within the experimental margin for determination.²⁹ For
 281 dense particles modified with EDTA, DTPA and TTHA ligands the uptake and
 282 release by treatment with 0.1 M HNO₃ was repeated up to 5 times without any
 283 measurable loss of capacity.²⁷



284
 285 **Scheme 1.** Chemical transformations in surface grafting of functional ligands (L) using EDTA as
 286 example onto Kromasil particles and subsequent adsorption and desorption of REE cations.

287 The white powder consisted of spherical particles with the size 4 -15 μm with statistic
 288 size distribution centered at 10 μm as reported in the industrial specification,
 289 according to the SEM data (Fig. 1A-D). The microspheres were nanostructured and
 290 built up by partly coalesced small silica particles of about 20-30 nm in size according
 291 to AFM studies (Fig. 1E-H. Additionally, a full resolution AFM image is provided in
 292 Fig. FS13). The microscopic measurements were not able to distinguish the
 293 difference between ligand coverage. Grafting of ligands was easy to trace with the
 294 help of FTIR data indicating formation of amide bonds (see Scheme 1, Figs FS1-
 295 FS4). The spectrum of material annealed at 500 °C and free from organic
 296 constituents was dominated by strong bands typical for dehydrated silica, i.e. 1090
 297 ($\nu_{\text{as}}(\text{Si-O-Si})$), 800 ($\nu(\text{Si-O-Si})$) and 460 ($\delta(\text{Si-O-Si})$) cm^{-1} with minor intensity bands

298 revealing presence of residual hydroxyl functions at 950 ($\nu_{as}(\text{Si-OH})$), and $\delta(\text{O-H})$ at
299 about 1640 cm^{-1} .



300

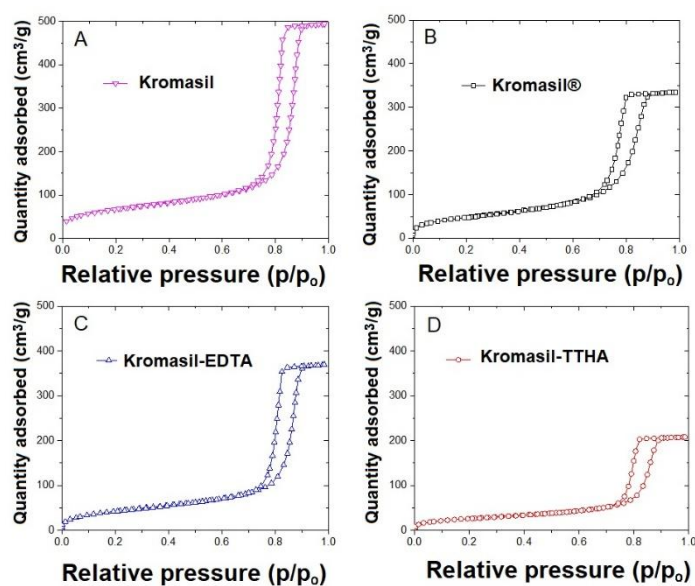
301 **Figure 1.** SEM and AFM images of annealed Kromasil (A, E), Kromasil® (B, F), EDTA-functionalized
302 Kromasil (C, G) and TTHA-functionalized Kromasil (D, H) respectively.

303 The spectra of initial Kromasil® and the samples modified with EDTA and TTHA
304 contained additionally protonated amine $\delta(\text{N-H})$ at about 1470 cm^{-1} (from the
305 attached APTES function). Grafting of the amino carboxylate ligands resulted in
306 appearance of several extra bands such as, in the first hand, at 1728 cm^{-1} ($\nu(\text{C=O})$
307 for carboxylic acid) and about 1397 cm^{-1} ($\delta(\text{O-H})$ of carboxylic acid). The
308 characteristic $\nu(\text{C-N})$ band observed usually at $1250\text{-}1020 \text{ cm}^{-1}$ was in this case
309 overlapping with intensity-saturated $\nu_{as}(\text{Si-O-Si})$ band (see Figs FS1-FS4).

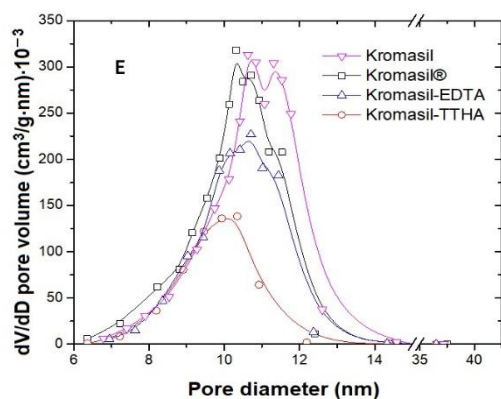
310 According to TGA data (see FS5-6), the content of organic components in Kromasil®
311 was 12-14 %, where covalently bound amino propyl ligands stayed for 9-9.5 % of
312 mass loss. The EDTA-functionalized sample loses adsorbed solvents below $180 \text{ }^\circ\text{C}$
313 (1.69 %) with subsequent dehydration and partial destruction, associated supposedly
314 with formation of anhydrides and amides and de-carboxylation in the temperature
315 interval $180\text{-}280^\circ\text{C}$ (16.23%). Decomposition with complete destruction of ligands

316 occurred in the broad temperature interval 280-500 °C (12.60%) and was finally
 317 followed by burning out of the residual organic carbon at over 500 °C (3.86 %). The
 318 total weight loss of the organic ligands was 32.66%, corresponding to grafted EDTA
 319 content of 0.85 mmol/g (See Fig. FS5). For the TTHA functionalized material, the
 320 loss of solvent (14.2 %) occurred below 130 °C and was followed by first the
 321 decomposition step at around 250 °C, associated with loss of 23.1 %, then multi-step
 322 destruction with the speed maximized at around 400 °C (16.5 %), and, finally, slow
 323 burnout of residual carbon at 500-665 °C (11.4 %). This corresponded to grafted
 324 TTHA content of 2.3 mmol/g (See Fig. FS6). The ligand contents in obtained
 325 adsorbents were verified by EDAX and elemental microanalysis, basing calculations
 326 on the determined nitrogen contents as characteristic for APCA ligands (See Fig.
 327 FS7, FS8 and Tab. TS2 and TS3).

328 The gas sorption data revealed very distinctly the mesoporous nature of all samples,
 329 as demonstrated by characteristic type IV isotherms with hysteresis loops all
 330 featuring the H1 shape, typical of cylindrical pores formed by uniform packing of
 331 spheres featuring essentially the same size (see Fig. 2A-D).⁴³



332



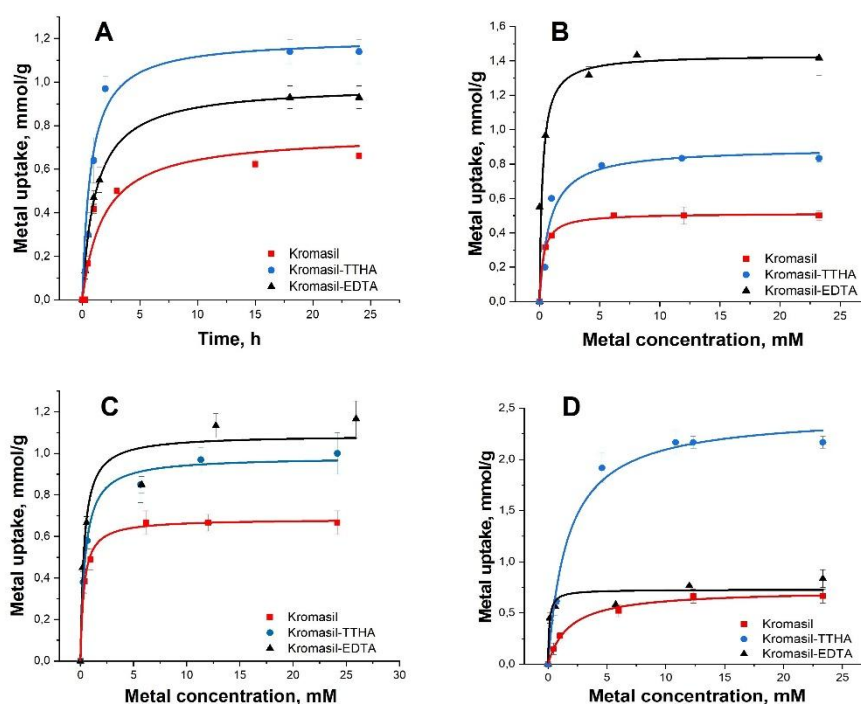
333

334 **Figure 2.** Nitrogen adsorption-desorption isotherms for annealed Kromasil (A), Kromasil® (B), EDTA-
335 functionalized Kromasil (C) and TTHA-functionalized Kromasil (D), and pore size distribution for all
336 samples (E).

337 This indicated that the actual shape of pores remained the same as observed by
338 AFM and implied uniform distribution of ligands on the surface, indicating grafting in
339 monolayer fashion. The measured active surface area was for annealed silica 243
340 m²/g with pore volume 0.764 cm³/g. The annealing lead apparently to some sintering
341 of the constituting 20-30 nm silica blocks, possessing originally additional
342 microporosity²⁹ (minor contribution with the size below cut-off limit in Fig. 2E) as for
343 APTES-bearing Kromasil® the surface area was 188 m²/g with somewhat smaller
344 pore volume 0.654 cm³/g. For the EDTA-functionalized material the surface area
345 was about 160 m²/g, pore volume 0.572 cm³/g, for DTPA (this work) 177 m²/g and
346 0.518 cm³/g, and for TTHA 97 m²/g and 0.322 cm³/g, respectively. This
347 corresponded well to the observed content of ligand by TGA: 0.85 mmol/g for EDTA,
348 just below 1 mmol/g for DTPA,²⁹ and 2.3 mmol/g for TTHA (Textural characteristics
349 of all materials were summarized in Supplementary Tab. TS1).

350 Investigation of the adsorption capacity at room temperature demonstrated that both
351 EDTA-functionalized Kromasil and TTHA-functionalized Kromasil MP, and even
352 Kromasil® were very efficient adsorbents. They all revealed quick kinetics of uptake
353 with about 60% of total capacity achieved already within 1 h and maximum capacity
354 approached in ca. 5 h (Fig. 3A). This was quite impressive for mesoporous materials
355 that might otherwise require several hours (up to 72) to achieve reasonable uptake
356 values for systems with small ordered mesopore structures.⁴ Functionalized
357 nanoparticles showed high adsorption capacity towards REE cations, with capacity
358 related to the nature of ligand and the cation size. As it had earlier been
359 demonstrated for monolayer complexes,²⁷⁻²⁸ formed on the surface of dense silica
360 particles, the ligands, possessing larger number of carboxylate groups, favored
361 larger REE cations. This resulted in higher capacity towards lighter REE for more
362 branched ligands.²⁷⁻²⁸ Thus Kromasil-TTHA revealed considerably higher maximum
363 adsorption capacity of 2.2 mmol/g towards La³⁺-cations compared to about 1.0
364 mmol/g for Nd³⁺ and only 0.83 mmol/g for Dy³⁺ (Fig. 3B-D, Tab. 1). For less
365 branched EDTA ligand the trend in adsorption capacity was reverse, i.e. 0.84 mmol/g
366 towards La³⁺-cations compared to about 1.2 mmol/g for Nd³⁺ and almost 1.4 mmol/g
367 for Dy³⁺ (see Tab. 1). The uptake by unmodified Kromasil® was essentially the same
368 and considerably lower for all three cations, in the range 0.5-0.67 mmol/g (see Fig.
369 FS9). The maximum capacity for all three adsorbents was evaluated by
370 complexometric titration and confirmed by EDAX mapping (see Figs. FS11, FS12).
371 Consistent with the correlation coefficient (R²) values, Langmuir model fitted the
372 adsorption process better than Freundlich model. The R² values for Langmuir model
373 in Nd³⁺ adsorption were 0.96 and 0.99 for Kromasil-EDTA and Kromasil-TTHA
374 nanoparticles respectively, compared to 0.92 and 0.97 for Freundlich model (see Fig.
375 FS 10).

376 Good fitting with Langmuir isotherm is generally indicative of the presence of uniform
 377 adsorption sites. In case of Kromasil-derived adsorbents in this work the adsorption
 378 capacity originated from combination of interaction with amino propyl ligands,
 379 complex formation with complexons and, possibly, even minor contribution from
 380 residual silanol functions as it had been envisaged earlier for DTPA-modified amino
 381 silica.^{29,44} The capacity of non-functionalized Kromasil® in this work (0.5-0.67
 382 mmol/g REE) was considerably higher than recently reported data for dense
 383 magnetic nanoparticles functionalized by amino propyl ligands.⁴⁵ The reason may be
 384 sought in, on one hand, smaller size of Kromasil building blocks (20-30 nm)
 385 compared to that of particles reported in ref.⁴⁵ and thus larger surface area and
 386 higher content of ligand in relation to mass of the adsorbent. On the other hand, the
 387 confinement in medium size mesopores could contribute to stability of the surface
 388 complexes. The content of amino functions on Kromasil® according to the literature²⁹
 389 and the results of TGA and elemental analysis in this work was about 1.8 mmol/g.
 390 The amount of REE cations adsorbed on Kromasil® was corresponding to 1/3 of the
 391 amount of ligands, pointing at formation of Ln(H₂NC₃H₆)₃-type complexes with amino
 392 groups.



393
 394 **Figure 3.** Kinetics of Nd³⁺-cation adsorption on Kromasil nanoparticles modified with amino
 395 polycarboxylate ligands (A) and adsorption isotherms of Nd³⁺(B), Dy³⁺(C), and La³⁺(D) cations fit by
 396 Langmuir isotherms.

397 This indicated that major contribution to the total adsorption capacity in modified
 398 Kromasil adsorbents was due to formation of 1:1 complexes with amino APCA-
 399 ligands. These results were also in agreement with earlier observations concerning
 400 DTPA modified Kromasil²⁹ and implied that complexation with these adsorbents

401 favored inner sphere complexation already at neutral pH, while for dense
 402 nanoparticles the major mechanism with APCA was the outer-sphere complexation
 403 associated with lower selectivity.²⁷ The most important novel feature was thus the
 404 observed difference in affinity, showing that EDTA and TTHA modified adsorbents
 405 possessed potentially more pronounced selectivity compared to the DTPA-modified
 406 one. The grafting of TTHA ligands, demonstrating enhanced affinity towards lighter
 407 REE, has not been reported so far to the best of our knowledge. The total capacity of
 408 the EDTA (this work) and DTPA²⁹ Kromasil based adsorbents lied in the range
 409 traced by Sillanpää et al. for heavy transition elements, Ni(II), Co(II) etc on APCA
 410 functionalized carbohydrate polymers.¹⁹⁻²¹ However, in their studies they were
 411 focusing on simpler amino-modified silica gels for adsorption of REE.⁴⁶ The observed
 412 trends in selectivity and uptake were also in line with earlier results from dense
 413 surface modified silica nanoparticles,^{22,26,27} but the capacity observed in the present
 414 work was considerably (50-100%) higher, especially for La³⁺ on TTHA grafted
 415 material. A remarkable feature of the adsorbents reported in the present work was
 416 also their outstanding chemical stability. In adsorption at pH 6.5 and desorption in
 417 0.1 M HNO₃ (pH = 1) the loss of capacity was within the standard deviation of the
 418 experiments in at least 50 adsorption-desorption cycles. An important advantage of
 419 the produced materials was also their hydrophilicity, apparently facilitating diffusion
 420 of aqueous solutions and permitting their recycling at more moderate pH, compared
 421 to macroporous silica composites with crown ether or calixcrown functionalized
 422 polymers. The latter were requiring 2-5 M HNO₃ for eluting Sr(II) and La(III).⁴⁷⁻⁴⁹
 423 Lower concentrations of nitric acid used in renewal of the adsorbent were
 424 contributing apparently to its better stability, advantageous for potential
 425 chromatographic applications as it was traced earlier for the DTPA-functionalized
 426 Kromasil.²⁹

427 **Table 1.** REE uptake by Kromasil® and EDTA and TTHA functionalized Kromasil (including Langmuir
 428 fitting parameters).

Adsorbent	Adsorption capacity, mmol/g; affinity, L/mg, R ²		
	Dy	Nd	La
Kromasil®	0.500 ± 0.05; 0.0052; 0.9993	0.667 ± 0.05; 0.0049; 0.9992	0.660 ± 0.05; 0.0022; 0.9969
Kromasil-EDTA	1.416 ± 0.15; 0.0049; 0.9996	1.166 ± 0.15; 0.0015; 0.9939	0.836 ± 0.05; 0.0021; 0.9970
Kromasil-TTHA	0.833 ± 0.05; 0.0018; 0.9970	1.041 ± 0.05; 0.0022; 0.9989	2.166 ± 0.05; 0.0013; 0.9946

429 For experimental details, please, see Figure FS14.

430 3.2 Biological experiments results

431 Humans are exposed to micro- and nanoparticles through different ways such as
 432 environmental contaminations, nano-remediation, spillage of nano- or micro-particles
 433 during industrial process, exposure from medical or consumer products, etc.
 434 Therefore, toxicological studies are of great importance.^{35, 50} The toxicological effect
 435 related to silica exposure, especially crystalline silica (0.5 – 10 µm), on humans has

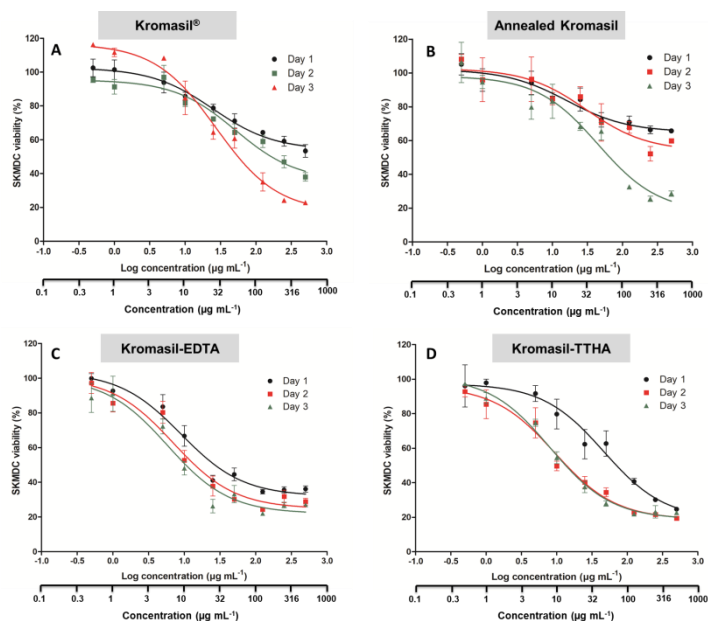
436 been reported.⁵¹⁻⁵² In 1997, the IARC (International Agency for Research on Cancer)
437 has classified crystalline silica as group 1 carcinogenic to human. Leung et al.
438 reported the silicosis as a pathogenic consequence of inhalation of free crystalline
439 silica in workers.^{53,54} Park et al. reported that the toxicity studies of amorphous
440 synthetic silica nano- or micro-particles were not done on large scale.⁵⁴

441

442 Micro- and nanoparticles are internalized into the human body *via* skin contact,
443 inhalation or ingestion, and subsequently, enter in contact with cells. Therefore, in
444 this work it was of utmost importance to study the toxicological effect of these MP on
445 the most representative cell populations encountered by the MP across these ways
446 of exposure. For MP studied in this work we saw as most probable the uptake *via*
447 gastro-intestinal tract and thus chose as models the macrophage cells that
448 represented the most common cells of the immune system, fibroblast cells that
449 represented the most common cells of the connective tissue, HUVECs that
450 represented the blood vessel cells and skeletal muscle-derived cells. In addition,
451 ecological risks of MP leakage into the water paths in the environment were further
452 tested in Zebrafish embryos.

453 Herein, cytotoxicity in human skeletal muscle-derived cells (SKMDCs) was evaluated
454 as a percentage of live cells, according to MTT assay, as a function of the dose and
455 the time of treatment. Results showed the classical sigmoidal dose-response curve
456 when plotted as a logarithmic function of MP concentration ($\mu\text{g mL}^{-1}$) versus the
457 percentage of viable cells as shown in Fig. 4A-D. In all figures, increasing the
458 concentration was associated with a decrease in the cell viability percentage
459 (concentration-dependent toxicity). Also, increasing the incubation time, from 1 to 3
460 days, was associated with an increase in cell mortality (time-dependent toxicity).

461 Calculations of the LC50 values for each MP at 50% of cell death are shown in Fig.
462 4E. After 1 day of treatment, Kromasil grafted with EDTA and TTHA MP were more
463 toxic, they exhibited lower LC50 values than Kromasil® and annealed Kromasil,
464 where the LC50 values could not be detected under these conditions ($>500 \mu\text{g mL}^{-1}$).
465 The LC50 values for all MP were decreased by days (Fig. 4E), which suggested
466 accumulative toxic effect. In fact, after 3 days of treatment, LC50 values were $13 \mu\text{g mL}^{-1}$,
467 $16 \mu\text{g mL}^{-1}$, $63 \mu\text{g mL}^{-1}$ and $71 \mu\text{g mL}^{-1}$ for Kromasil grafted with EDTA,
468 Kromasil grafted with TTHA, Kromasil® and annealed Kromasil, respectively. All
469 these results suggested the lower toxicity, in order, of annealed Kromasil followed by
470 Kromasil®, Kromasil grafted with TTHA then Kromasil grafted with EDTA.



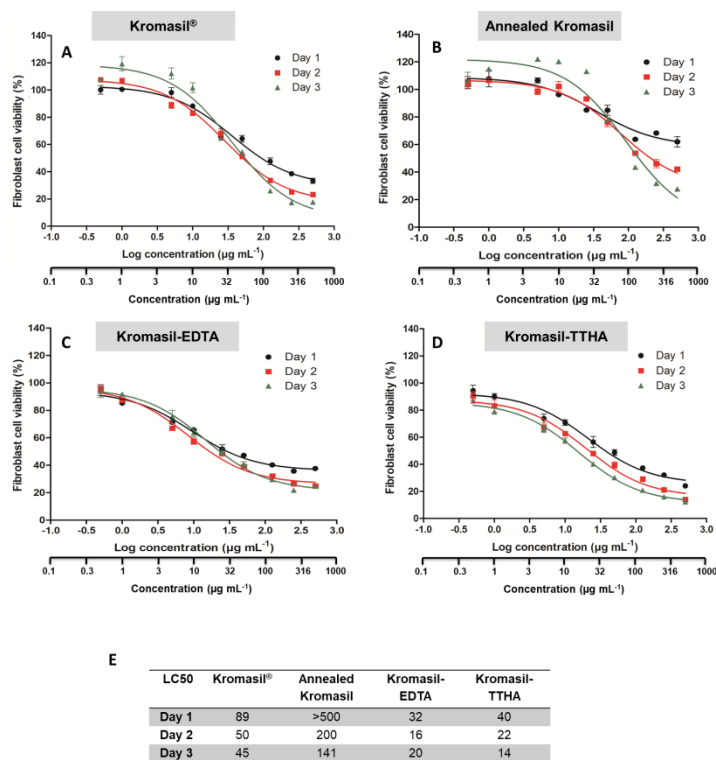
E

	LC50 Kromasil®	Annealed Kromasil	Kromasil-EDTA	Kromasil-TTHA
Day 1	>500	>500	35	79
Day 2	178	500	18	18
Day 3	63	71	13	16

471

472 **Figure 4.** Dose-response curves representing the cell viability percentage (%) of SKMDCs treated
 473 with different concentrations of Kromasil® (A), annealed Kromasil (B), EDTA-functionalized Kromasil
 474 (C) and TTHA-functionalized Kromasil (D) for 1, 2 and 3 days. Results are presented as mean ± SEM,
 475 n=3. (E) LC50 values (as $\mu\text{g mL}^{-1}$) for all MP in SKMDCs.

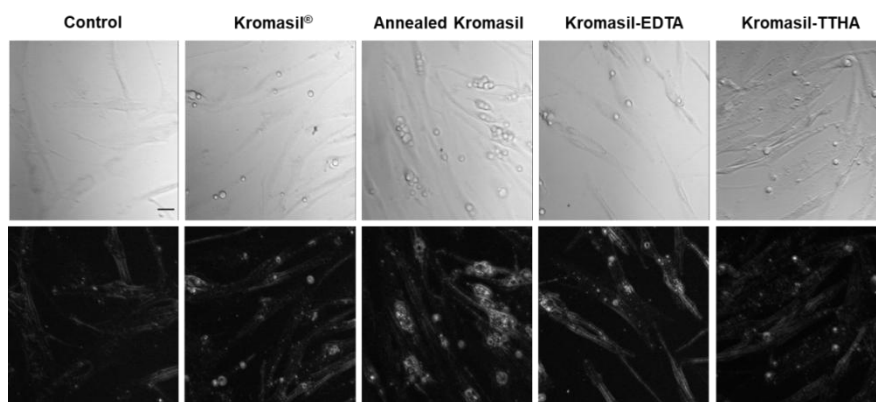
476 Fibroblast cells were used as the most common cell type of connective tissue, the
 477 classical sigmoidal dose-response curves for all MP after 1, 2 and 3 days of
 478 treatment are shown in Fig. 5A-D, in which time- and concentration-dependent
 479 effects were observed. The LC50 values for each type of MP are shown in Fig. 5E,
 480 after 1 day of treatment, the higher LC50 value was observed for annealed Kromasil
 481 ($\text{LC50} >500 \mu\text{g mL}^{-1}$) in compared to other MP, confirming their lower cytotoxic
 482 effect. After three days of treatment, results showed that the annealed Kromasil MP
 483 induced lower cytotoxic effect ($\text{LC50} = 141 \mu\text{g mL}^{-1}$) than Kromasil® ($\text{LC50} = 45 \mu\text{g mL}^{-1}$),
 484 Kromasil grafted with EDTA ($\text{LC50} = 20 \mu\text{g mL}^{-1}$) and Kromasil grafted with
 485 TTHA MP ($\text{LC50} = 14 \mu\text{g mL}^{-1}$).



486

487 **Figure 5.** Dose-response curves representing the cell viability percentage (%) of fibroblast cells
 488 treated with different concentrations of Kromasil® (A), annealed Kromasil (B), EDTA-functionalized
 489 Kromasil (C) and TTHA-functionalized Kromasil (D) for 1, 2 and 3 days. Results are presented as
 490 mean \pm SEM, n=3. (E) LC50 values (as $\mu\text{g mL}^{-1}$) for all MP in fibroblast cells.

491 The internalization of the MP inside the cells was tested as shown in Fig. 6.
 492 Fibroblast cells were treated with a concentration lower than LC50 ($25 \mu\text{g mL}^{-1}$),
 493 results showed an obvious internalization of the MP into the cells. Internalization of
 494 MP was already observed in various studies, Serda et al. demonstrated the efficient
 495 internalization of nanoporous silicon MP (1.6 and $3.2 \mu\text{m}$) into HUVECs with no
 496 effect on cellular integrity.⁵⁵ Also, Bimbo et al. showed the internalization of porous
 497 silicon MP (1-10 μm) in RAW264.7 macrophage cells.⁵⁶

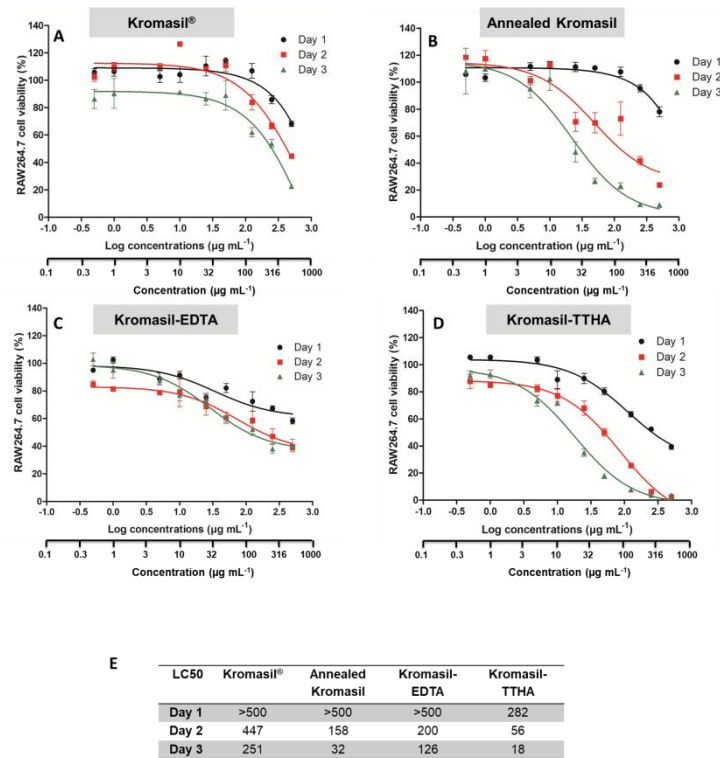


498

499 **Figure 6.** Z-stacking images of living fibroblasts not treated or treated with $25 \mu\text{g mL}^{-1}$ of different MP
 500 for 24 h. Scale bar 30 μm .

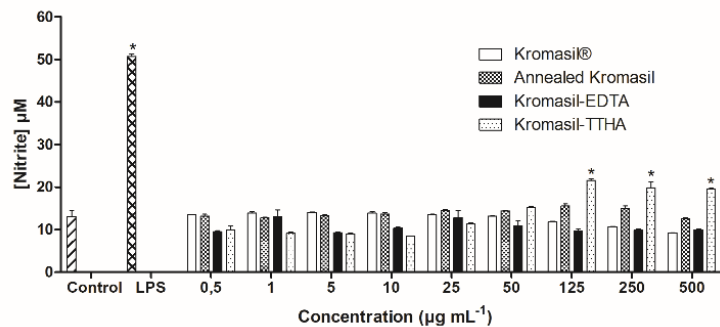
501 Macrophages are professional phagocytes with the ability to uptake any foreign
502 particulates introduced to the body, including the exposure *via* gastro-intestinal tract.
503 Inhaled MP caught in the nose or upper respiratory tract were demonstrated to be
504 eliminated by macrophages or mucociliary escalator; therefore evaluation of the toxic
505 effect of MP on macrophages was important to estimate the overall toxicity.⁵⁷ Here
506 we studied RAW264.7 cell viability when incubated with increasing concentrations of
507 MP during 1, 2 and 3 days (Fig. 7A-D). The LC50 values for each MP are shown in
508 Fig. 7E. After 1 day of treatment, the LC50 values for all MP were above 250 $\mu\text{g mL}^{-1}$.
509 These results indicated the low cytotoxic effect of all MP on macrophages in
510 comparison with SKMDCs and fibroblast cells after 1 day of treatment. Then toxicity
511 increased with the incubation time, and demonstrated that Kromasil® was less toxic,
512 in order, than Kromasil grafted with EDTA, annealed Kromasil and Kromasil grafted
513 with TTHA.

514 Oxidative stress is one of the mechanisms responsible for the cytotoxicity. Oxidative
515 stress induced by nano- or MP enhances inflammation through upregulation of
516 redox-sensitive transcription factors, including nuclear factor kappa B and activated
517 protein 1, which induce mRNA expression of proinflammatory mediators, and finally
518 cause inflammation.⁵⁶ Several studies showed the close relationship between
519 reactive oxygen species production and nitric oxide (NO), a pro-inflammatory
520 mediator that induced inflammation and a marker of oxidative stress,⁵⁸ release in
521 RAW264.7 macrophage cells after incubation with nano- or micro-particles.⁵⁴ For
522 this, the extracellular measurement of NO was analyzed using Griess reagent as a
523 total nitrite concentration. Results reported in Fig. 8 showed that macrophages
524 exhibited a significant increase in NO level when treated with Kromasil grafted with
525 TTHA at a concentration of 125 $\mu\text{g mL}^{-1}$ and above, which explained the obtained
526 toxicity on this cell line. In case of annealed Kromasil, although it showed no
527 induction of NO, after 1 day, an increase in the toxicity effect with time was
528 observed, suggesting that oxidative stress was not the responsible mechanism of
529 cell death. However, a defect in the cytoskeleton could be the cause⁵⁹ due to higher
530 cellular uptake of these MP as observed in Figure 6.



531

532 **Figure 7.** Dose-response curves representing the cell viability percentage (%) of RAW264.7 cells
 533 treated with different concentrations of Kromasil® (A), annealed Kromasil (B), EDTA-functionalized
 534 Kromasil (C) and TTHA-functionalized Kromasil (D) for 1, 2 and 3 days. Results are presented as
 535 mean \pm SEM, n=3. (E) LC50 values (as $\mu\text{g mL}^{-1}$) for all MP in RAW264.7 cells.

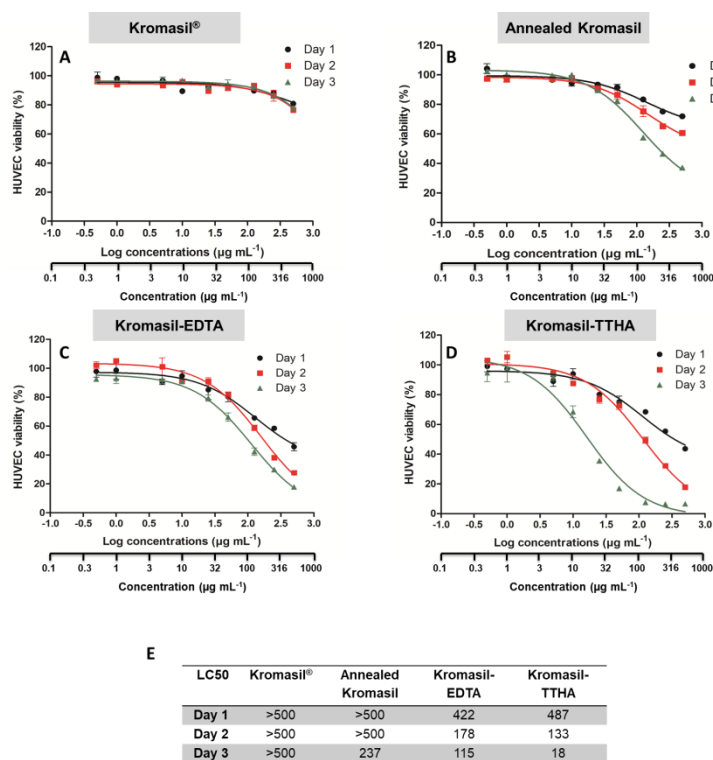


536

537 **Figure 8.** Extracellular measurement of nitrite concentration presents in the supernatant of RAW264.7
 538 macrophages growth medium using Griess assay. Results are presented as mean \pm SEM, n=3.
 539 Statistical analysis carried out using unpaired t-test. * Statistically significant difference from control
 540 ($P < 0.05$).

541 To study the toxicological behavior of these MP on the endothelial cell function,
 542 HUVECs cell line was used, dose-response curves of HUVECs are shown in Fig.
 543 9A-D. LC50 values were above $400 \mu\text{g mL}^{-1}$ for all MP after 1 day of treatment (Fig.
 544 9E). The LC50 values for Kromasil® did not change by days ($\text{LC50} > 500 \mu\text{g mL}^{-1}$) in
 545 these conditions. After 3 days of treatment, the LC50 values were $237 \mu\text{g mL}^{-1}$, 115
 546 $\mu\text{g mL}^{-1}$ and $18 \mu\text{g mL}^{-1}$ for annealed Kromasil, Kromasil grafted with EDTA and
 547 Kromasil grafted with TTHA, respectively. These results suggested low cytotoxic

548 behavior of MP in HUVECs in comparison with SKMDCs, fibroblast cells and
 549 macrophages (cell-type dependent toxicity).

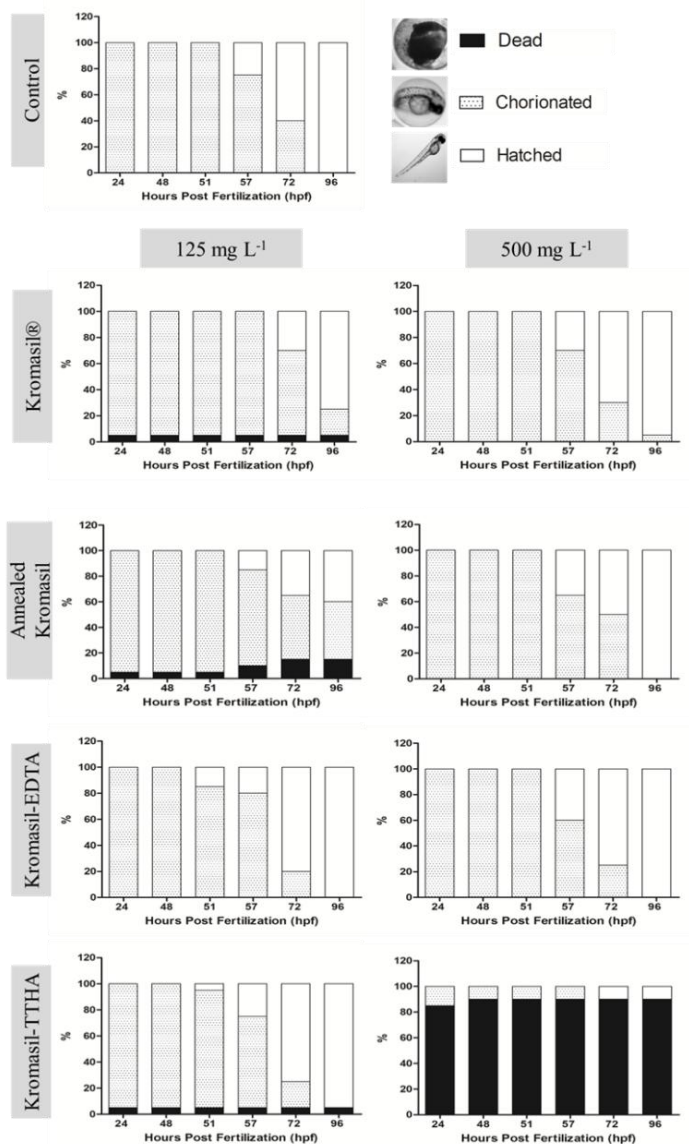


551 **Figure 9.** Dose-response curves representing the cell viability percentage (%) of HUVECs treated
 552 with different concentrations of Kromasil[®] (A), annealed Kromasil (B), EDTA-functionalized Kromasil
 553 (C) and TTHA-functionalized Kromasil (D) for 1, 2 and 3 days. Results are presented as mean \pm SEM,
 554 $n=3$. (E) LC50 values (as $\mu\text{g mL}^{-1}$) for all MP in HUVECs.

555 From all the above results, we could conclude that the cytotoxic behavior of the MP
 556 was concentration-, time-, cell- and ligand-dependent. An increase in toxicity was
 557 noted with the increase in microparticle concentration and time of incubation. In
 558 addition, HUVECs showed lower sensitivity toward the MP in compared to other cell
 559 lines. Finally, grafting APCA to Kromasil MP increased the cytotoxicity; it was evident
 560 that Kromasil grafted with TTHA was more toxic in all cell lines in compared to other
 561 MP, with the ability to induce inflammation in macrophages indicated by an increase
 562 in the NO level. Cytotoxicity was due to the uptake of the MP by the cells, which
 563 subsequently destabilize the cellular machinery owing to their large size.

564 Nano- or micro-particles will discard in the aquatic system in the form of wastewater
 565 or effluents.^{60,61} Therefore, their environmental impact must be addressed. *In vivo*
 566 toxicity of MP was evaluated in zebrafish embryos. Gastrula stage embryos were
 567 exposed to water containing 0 or 125 or 500 mg L^{-1} of MP. Percentages of
 568 chorionated, dead and hatched embryos were calculated at different time points (24,
 569 48, 51, 56, 72 and 96 hpf). Results showed that Kromasil[®], annealed Kromasil and
 570 Kromasil grafted with EDTA had no toxic effect on embryos after 96 hpf of exposure

571 to 125 and 500 mg L⁻¹ of MP, with no changes in motility, morphology and hatching
 572 rate in compared to control (Fig. 10).



573

574 **Figure 10.** Zebrafish embryos development, expressed as percentages of dead, chorionated and
 575 hatched, in water containing different concentrations of MP for 24, 48, 51, 57, 72 and 96 hpf .

576 In case of Kromasil grafted with TTHA, exposing the embryos to 125 mg L⁻¹ showed
 577 low toxicity with no changes in motility, morphology and hatching rate in compared to
 578 control. In contrast, at 500 mg L⁻¹, 85% of mortality was observed at 24 hpf embryos,
 579 which reached to 90% after 96 hpf.

580 4. CONCLUSIONS

581 APCA ligand grafted Kromasil particles could easily be produced by a facile general
 582 approach, exploiting formation of an amide bond. The grafting left the attached
 583 ligand functional in adsorption of REE cations. Produced adsorbents revealed high
 584 and element-selective capacity in the range 0.83-2.2 mmol/g and could be attractive

585 for applications in hydrometallurgy and recycling of REE. Cytotoxicity studies
586 showed results that were concentration-, time-, cell- and ligand-dependent. The
587 toxicity of ligand-grafted particles was appreciably higher, especially for Kromasil-
588 TTHA, than that of ligand-free (annealed Kromasil) or aminopropyl functionalized
589 particles (Kromasil®). Internalization of the MP inside the cells was observed. These
590 data were correlated with those obtained *In vivo*. Toxicity studies in Zebrafish
591 embryos showed no toxicity for all MP, except Kromasil-TTHA, at high concentration
592 (500 mg L⁻¹) after 96 hpf.

593 ASSOCIATED CONTENT

594 Supporting Information. FTIR, TGA, EDS mapping results for all samples, and fitting
595 of adsorption isotherm of Nd³⁺-cations on Kromasil-EDTA and Kromasil-TTHA
596 samples by Freundlich equation.

597 Author Contributions

598 The manuscript was written through contributions of all authors. All authors have
599 given approval to the final version of the manuscript. ‡These authors contributed
600 equally.

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606

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611

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- 794

795 **Supplementary materials**

796 **Mesoporous silica adsorbents modified with amino polycarboxylate** 797 **ligands – functional characteristics, health and environmental** 798 **effects**

799 Gulaim A. Seisenbaeva,^a Lamiaa M.A. Ali,^{b,c,d§} Ani Vardanyan,^{a§} Magali Gary-Bobo^d,
800 Tetyana Budnyak,^{e,f} Vadim G. Kessler,^a Jean-Olivier Durand^b

801 a Department of Molecular Sciences, BioCenter, Swedish University of Agricultural
802 Sciences, Box 7015, SE-75007, Uppsala, Sweden

803 b Institut Charles Gerhardt de Montpellier, UMR5253, Université de Montpellier, Place
804 EugèneBataillon, CC 1700 – Bâtiment 17, 34095 Montpellier Cedex 5, France

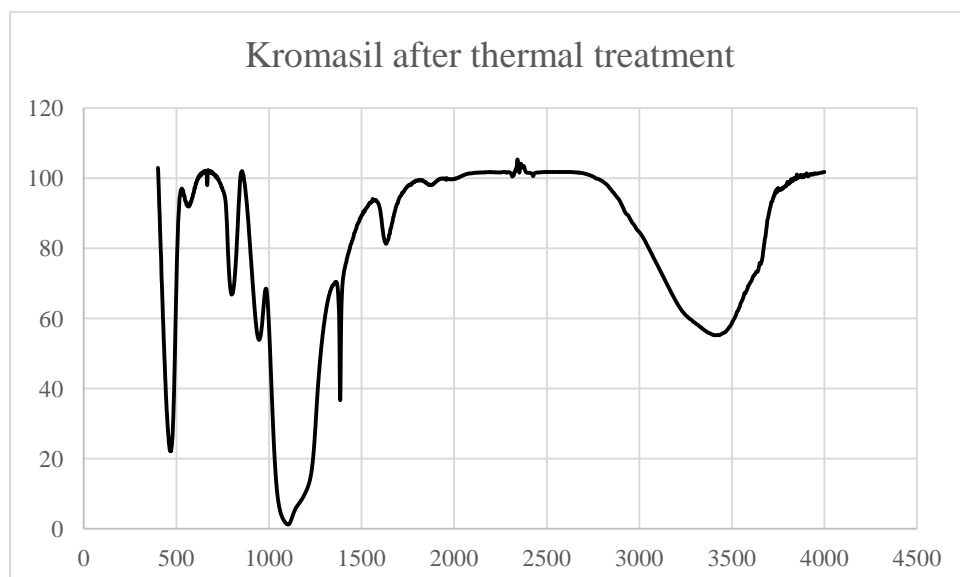
805 c Department of Biochemistry, Medical Research Institute, University of Alexandria, 21561
806 Alexandria, Egypt.

807 d IBMM, Institut des Biomolécules Max Mousseron, UMR 5247 CNRS, UM-Faculté de
808 Pharmacie, 15 Avenue Charles 9 Flahault, 34093 Montpellier Cedex 05 (France)

809 e Department of Materials and Environmental Chemistry, Stockholm University, Svante
810 Arrhenius väg 16 C, 106 91 Stockholm, Sweden

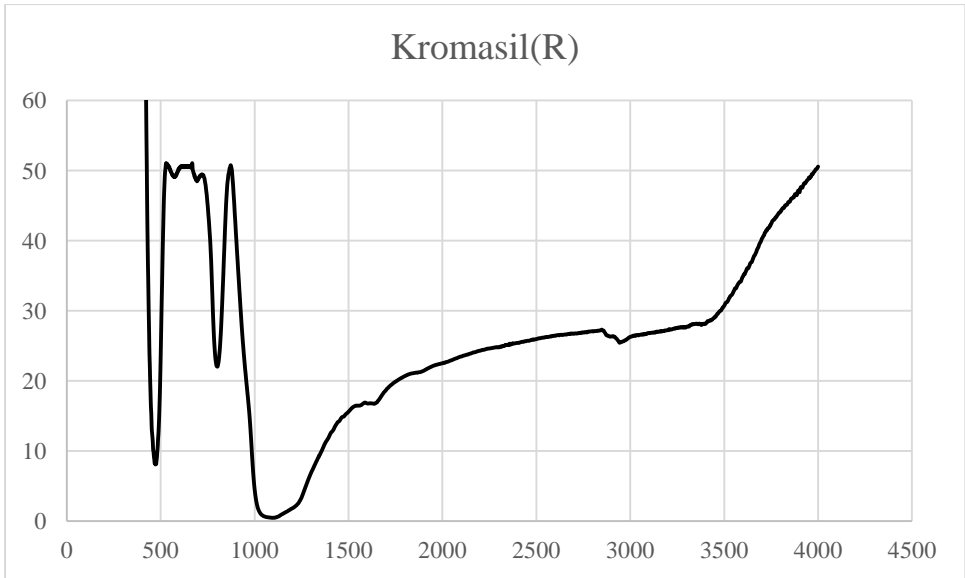
811 f Chuiko Institute of Surface Chemistry of National Academy of Sciences of Ukraine, 17
812 General Naumov Str., 03164 Kyiv, Ukraine

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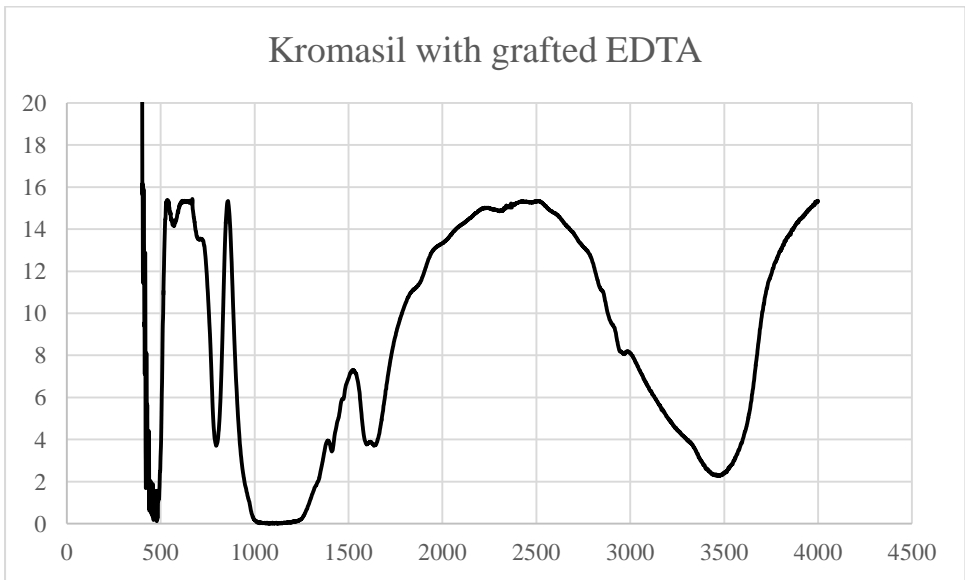
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815 Fig. FS1 FTIR spectrum of Kromasil® thermally treated at 500°C 1h for removal of organic
816 ligands.



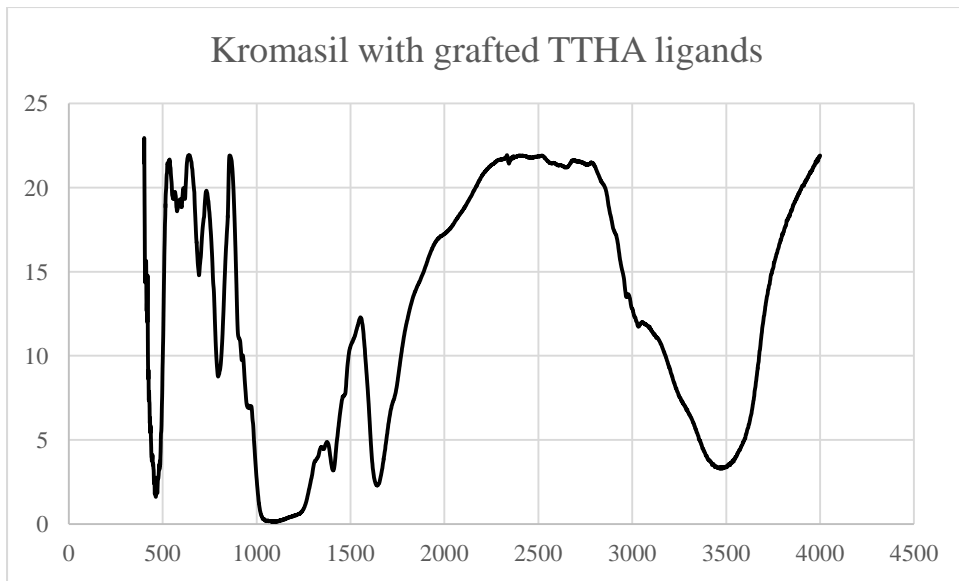
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818 Fig. FS2 FTIR spectrum of Kromasil® bearing amino propyl ligands.



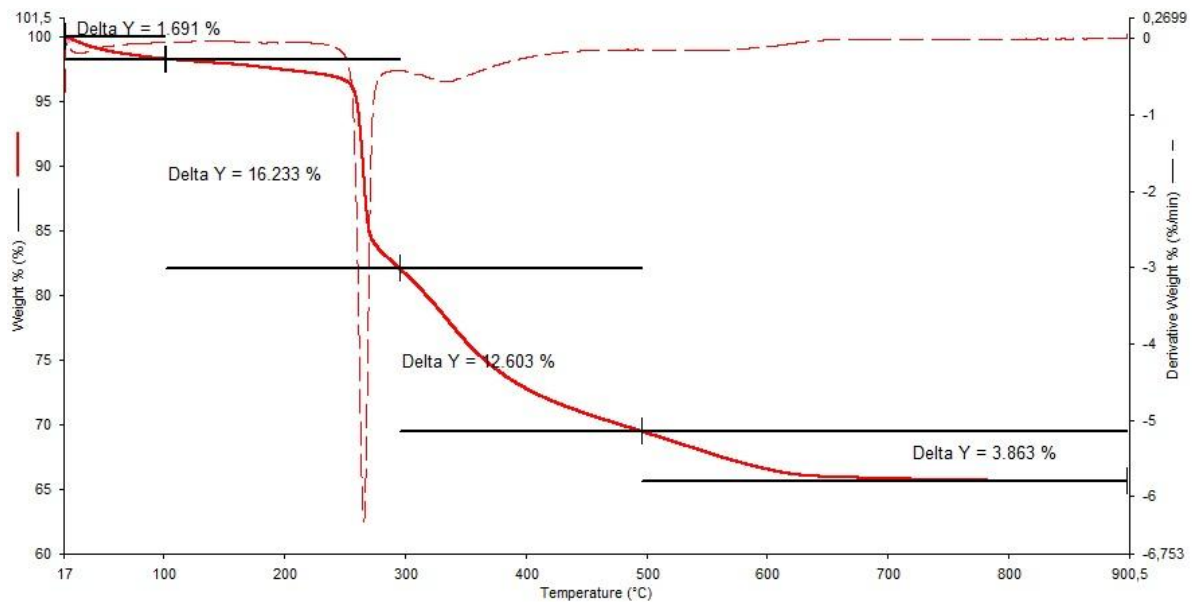
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820 Fig. FS3 FTIR spectrum of Kromasil® modified with EDTA ligands.



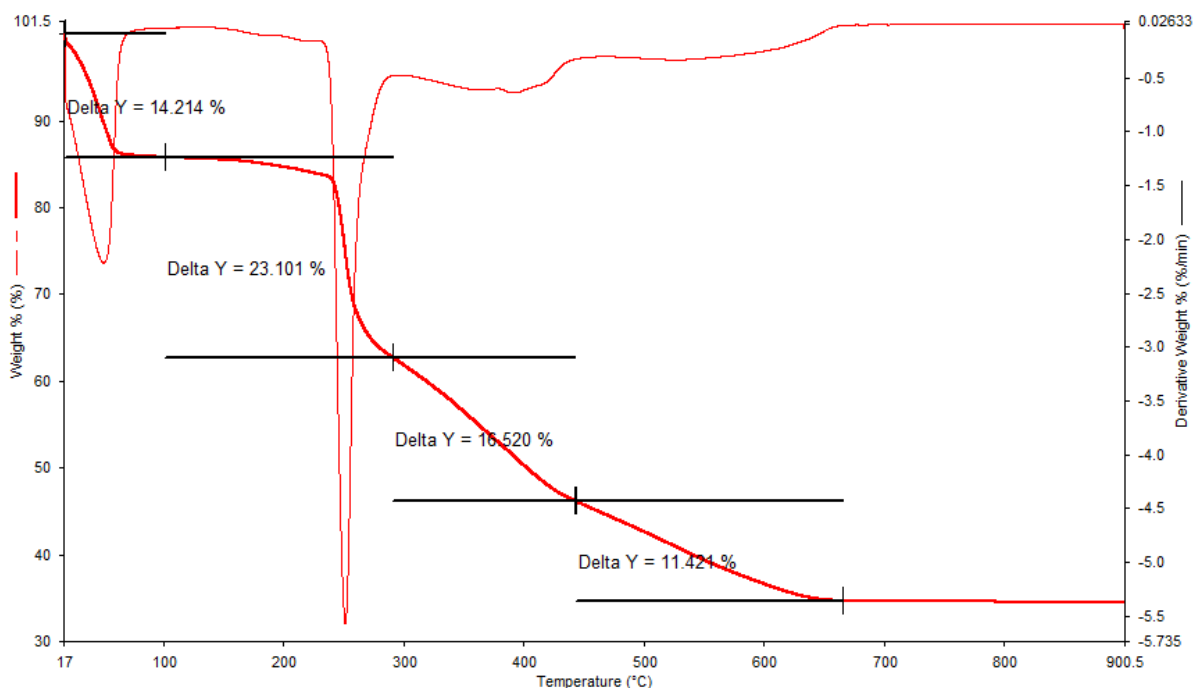
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822 Fig. FS4 FTIR spectrum of Kromasil® modified with TTHA ligands.



823

824 Fig. FS5 TGA of Kromasil® modified with EDTA ligands



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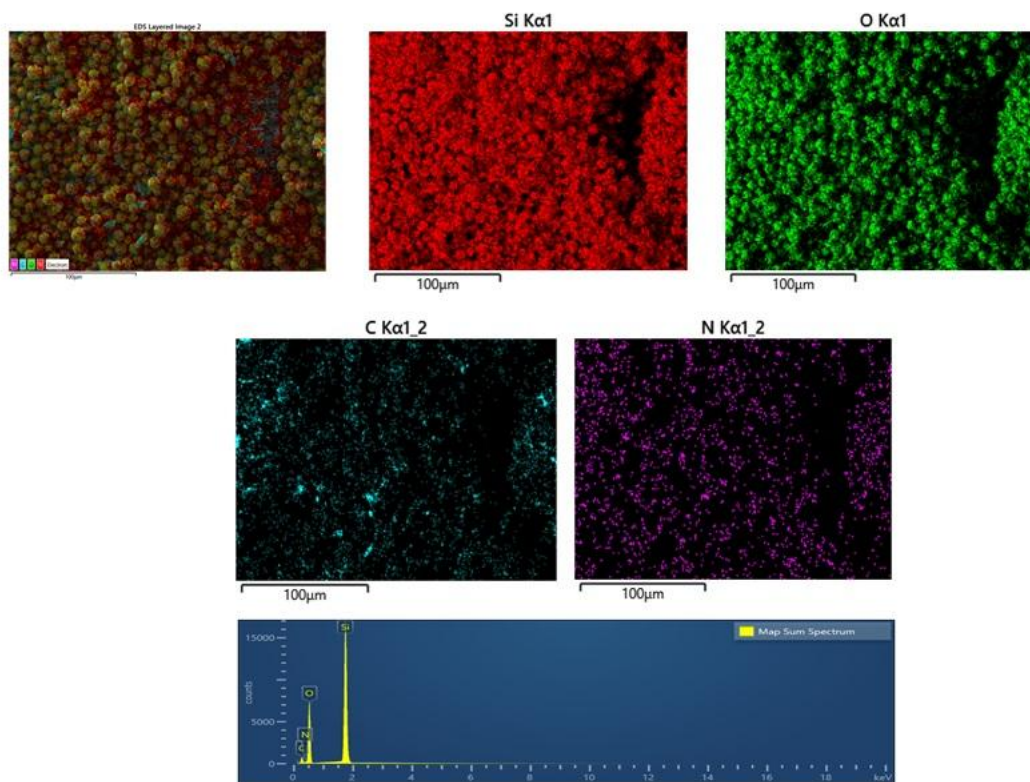
826 Fig. FS6. TGA of Kromasil® modified with TTHA ligands

827 Table TS1 Textural characteristics of materials.

Sample	S_{BET} , m^2/g	V_{pores} , cm^3/g	D_{pores} , nm
Kromasil	243	0.764	10.6
Kromasil®	188	0.654	10.0
Kromasil-EDTA	160	0.572	10.2
Kromasil-DTPA	177	0.518	8.6
Kromasil-TTHA	97	0.322	9.7

828 Note: S_{BET} – the Brunauer-Emmet-Teller (BET) surface area; V_{pores} – total pore volume; D_{pores}
 829 – BJH average pore diameter.

830 The curves of pore-size distribution by volume were obtained using the Barret-Joyner-
 831 Halenda (BJH) method [E.P. Barrett, L.G. Joyner, P.P. Halenda, The determination of pore
 832 volume and area distributions in porous substances. I. Computations from nitrogen
 833 isotherms, J. Am. Chem. Soc., 73 (1951) 373-380.] by desorption branch of the isotherms for
 834 all studied materials presented on Fig. Y. According to the IUPAC classification [W.
 835 Holleman, Lehrbuchde Anorganischen Chemie, Walter de Gruyter, Berlin, New York, 1995.]
 836 and obtained pore-size distribution (Fig. Y), all synthesized materials belong to mesoporous
 837 materials with diameter in the range from 6 – 15 nm.



Kromasil_EDTA

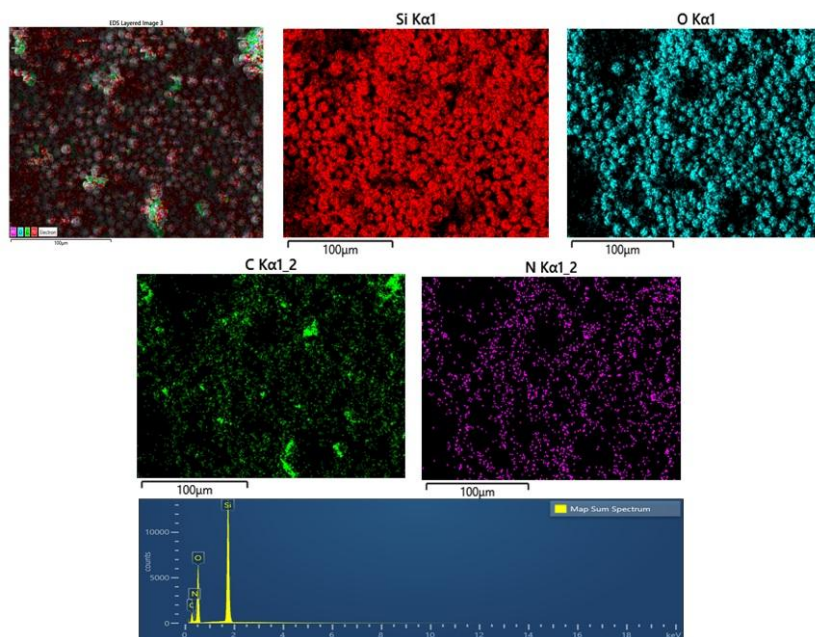
838

839 Fig. FS7. Element mapping and average X-ray emission spectrum for the Kromasil®
 840 modified with EDTA ligands

841 Table TS2 EDAX analysis of the Kromasil® modified with EDTA ligands

Map Sum Spectrum				
Element	Line Type	Weight %	Weight % Sigma	Atomic %
O	K series	48.87	0.39	53.48
Si	K series	32.63	0.27	20.34
C	K series	14.70	0.45	21.44
N	K series	3.80	0.47	4.75
Total		100.00		100.00

842



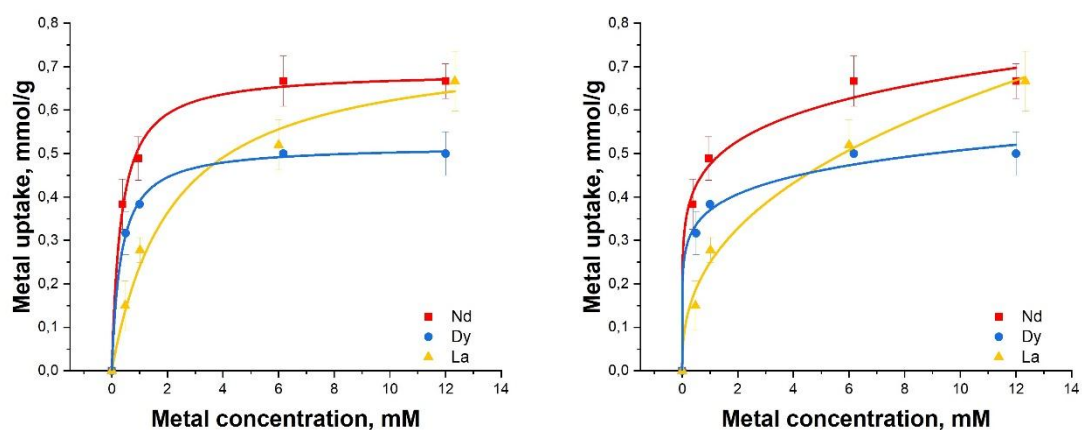
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844 Fig. FS8. Element mapping and average X-ray emission spectrum for the Kromasil®
 845 modified with TTHA ligands

846 Table TS3 EDAX analysis of the Kromasil® modified with TTHA ligands

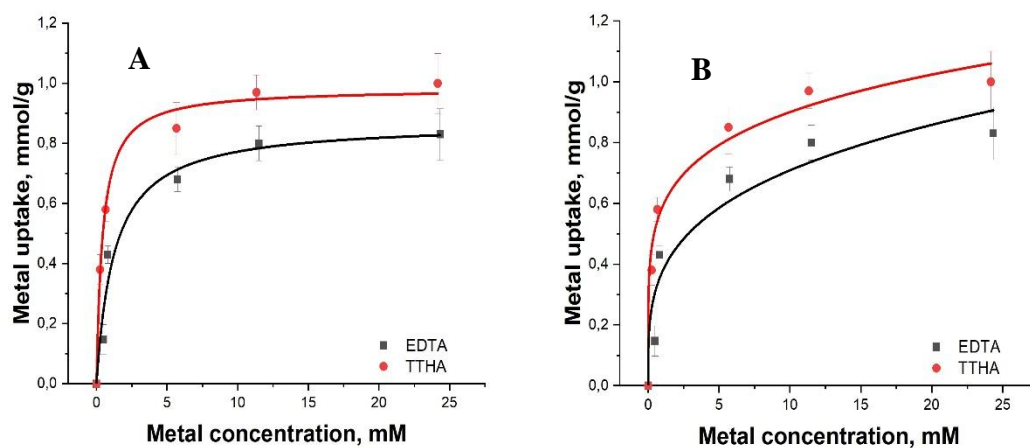
Map Sum Spectrum				
Element	Line Type	Weight %	Weight % Sigma	Atomic %
O	K series	47.32	0.43	49.53
Si	K series	27.49	0.26	16.39
C	K series	19.98	0.46	27.85
N	K series	5.21	0.55	6.22
Total		100.00		100.00

847



848

849 Fig. FS9 REE adsorption isotherms by Kromasil® MP. Langmuir isotherms – upper
 850 image, Freundlich isotherms – lower one.

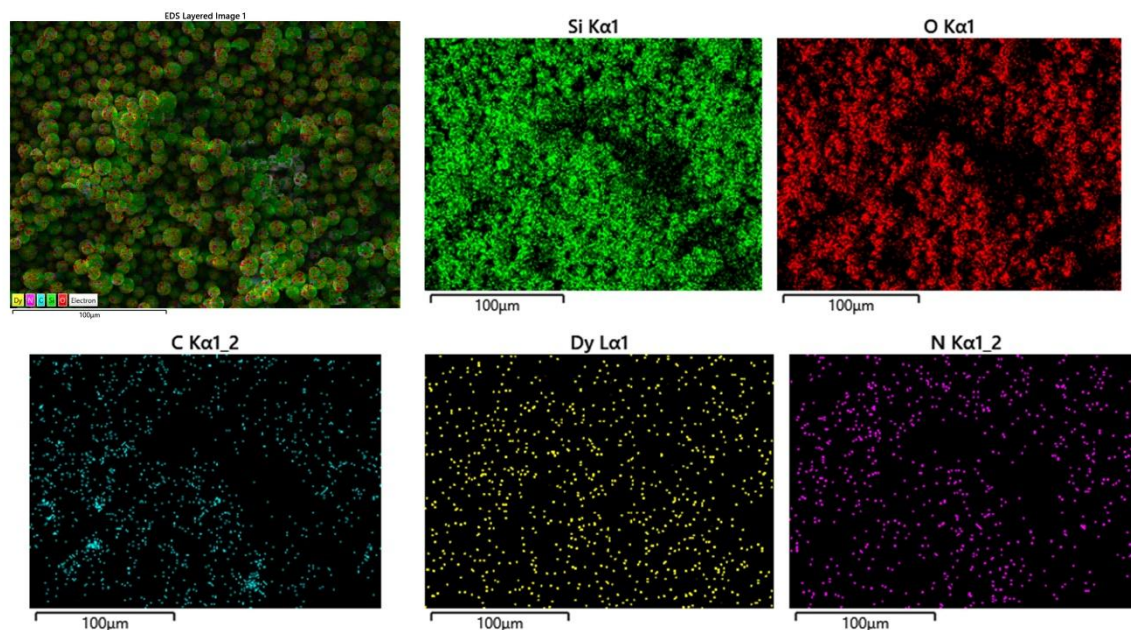


851

852 Fig. FS10 Adsorption isotherms of Nd^{3+} on Kromasil-derived adsorbents modified
 853 with amino polycarboxylate ligands: A-Langmuir isotherms, B-Freundlich isotherms.

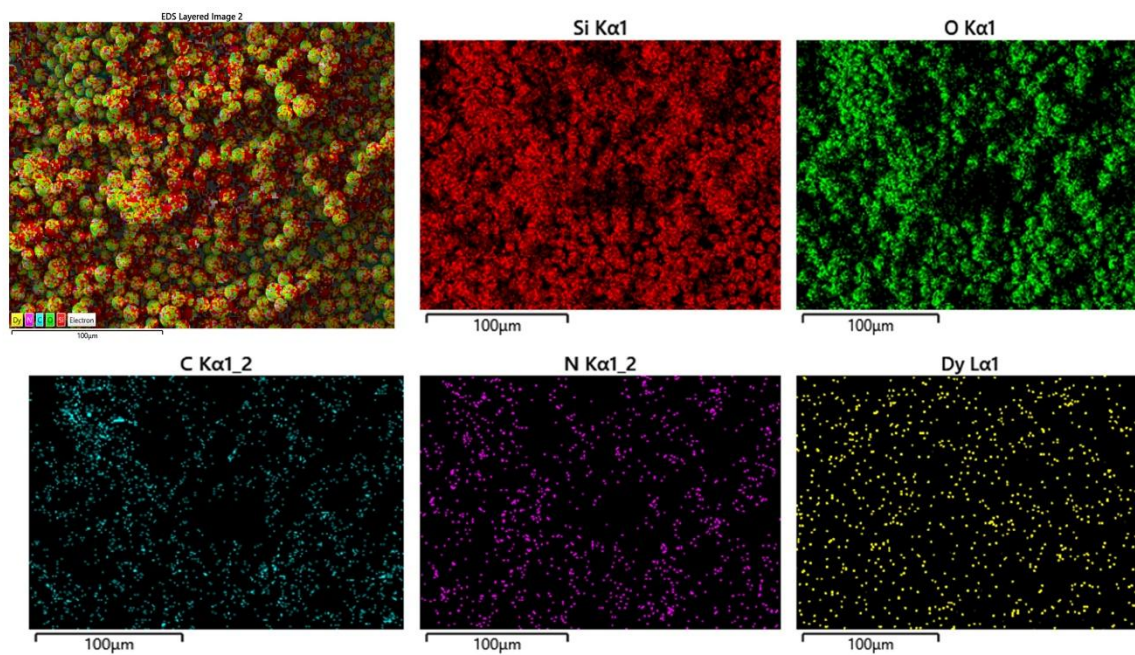
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856

857 Fig. FS11 EDS mapping of Kromasil® modified with EDTA ligands after uptake of Dy^{3+}
 858 cations.

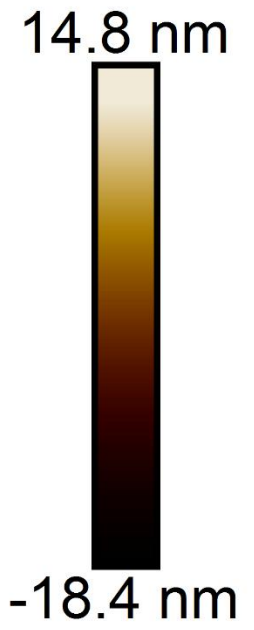
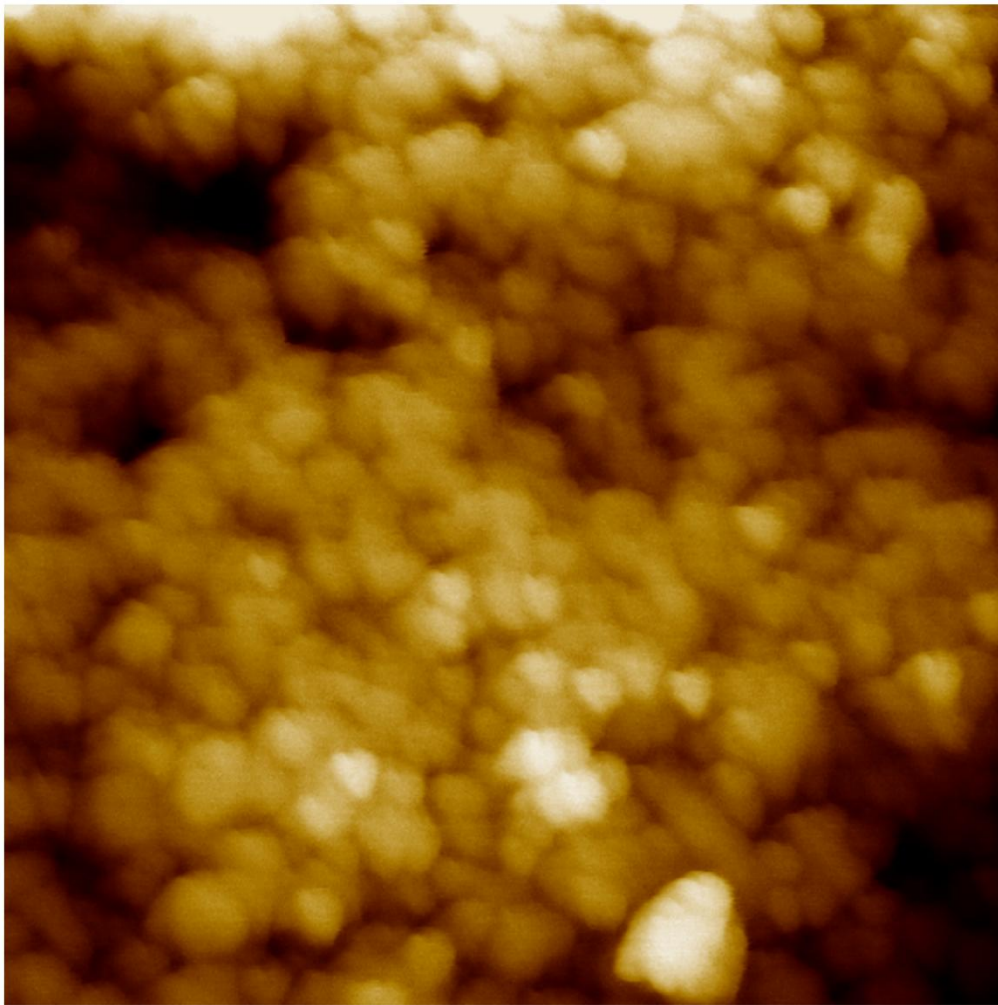


859

860 Fig. FS12 EDS mapping of Kromasil® modified with TTHA ligands after uptake of Dy³⁺
 861 cations.

862

863



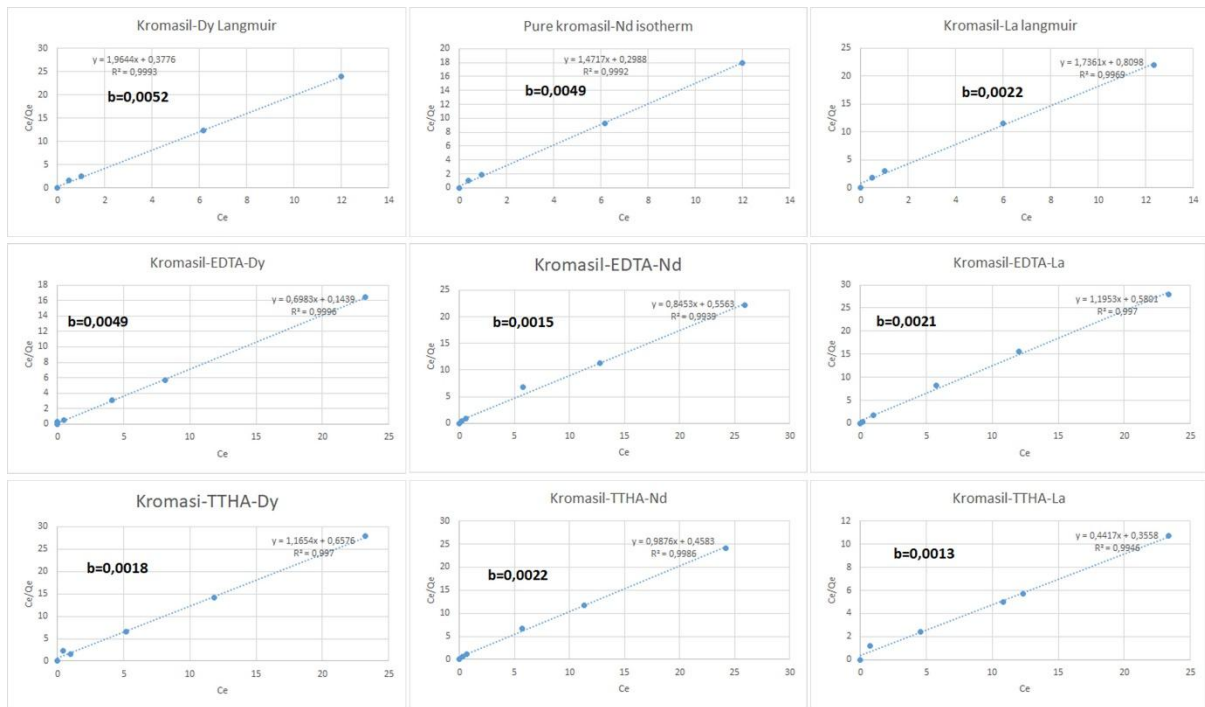
Height Sensor 100.0 nm

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Fig. FS13 Representative high resolution AFM image of Kromasil showing the partly fused 20-30 nm nanoparticles building up the Kromasil MP.



867

868 Figure FS14. Linearized plots of Langmuir adsorption isotherms (b -value is given in
869 L/mg units).