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Article

High-Purity Corundum as Support for Affinity Extractions from Complex Samples

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Abstract: Nonporous corundum powder, known as an abrasive material in the industry, was functionalized covalently with protein binders to isolate and enrich specific proteins from complex matrices. The materials based on corundum were characterized by TEM, ESEM, BET, DLS, and zeta potential measurements. The strong Al-O-P bonds between the corundum surface and amino phosphonic acids are used to introduce functional groups for further conjugations. The common cross-linker glutaraldehyde was compared with a hyperbranched polyglycerol (PG) of around 10 kDa. The latter is oxidized with periodate to generate aldehyde groups that can covalently react with the amines of the surface and the amino groups from the protein via a reductive amination process. The amount of bound protein was quantified via aromatic amino acid analysis (AAAA). This work shows that oxidized polyglycerol can be used as an alternative to glutaraldehyde. With polyglycerol, more of the model protein bovine serum albumin (BSA) could be attached to the surface under the same conditions, and lower nonspecific binding (NSB) was observed. As a proof of concept, IgG was extracted with protein A from crude human plasma. The purity of the product was examined by SDS-PAGE. A binding capacity of 1.8 mg IgG per g of corundum powder was achieved. The advantages of corundum are the very low price, extremely high physical and chemical stability, pressure resistance, favorable binding kinetics, and flexible application.

Keywords: protein affinity enrichment; bioseparation; immunoprecipitation; immunocapture; affinity chromatography; solid phase; carrier; material; corundum; polyglycerol; aromatic amino acid analysis; self-assembled monolayers (SAM), periodate oxidation; reductive amination; antibodies; IgG; immunoglobulins; glutaraldehyde; polyglycerol; hyperbranched polymer

1. Introduction

The demand for methods for the isolation of specific proteins from biological samples has significantly increased in recent years. Biological samples such as human blood, cell cultures for recombinant protein expression, or many raw materials containing proteins of interest are very complex in their composition. Therefore, their analysis and workup are often very challenging. The isolation of biomarkers from blood, which is usually performed via affinity enrichment, can be the bottleneck in detecting proteins of low abundance.[1,2] Proteins of higher concentrations, such as antibodies in plasma or cell cultures, need to be purified to isolate and enrich the protein on a larger scale.[3] These applications have become more and more critical for therapeutic antibodies, which are produced recombinantly for medicinal purposes.[4] Many platforms, column- or particle-based, can be used for protein enrichment, all with pros and cons. Materials like agarose or dextran-based polymers such as Sephadex can be used to capture and enrich proteins. [5-7] These materials are often used in low-pressure chromatographic systems (FPLC). Usually, pumps, columns, and a detection system are required to identify and isolate the enriched

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proteins properly. In contrast, protocols with magnetic beads are independent of a complex instrument setup and can be easily performed even in moderately equipped laboratories. When it comes to larger-scale enrichment, these particles are often too cost-intensive and therefore not frequently used.

The corundum particles used in this work are based on common abrasives for industrial purposes.[8-10] They show very high chemical and mechanical stability and are available in large amounts at very low prices in the range of 1-2 EUR/kg. Chemically, corundum is an aluminum oxide (α-Al_2O_3) and is produced by melting purified aluminum oxide in an electric furnace. The mineral bauxite is the raw material that has to be purified by the Bayer process.[11] Corundum belongs to the group of oxides, such as titanium, silicon, or iron oxides. In literature, many methods report the functionalization of surfaces of metal oxides.[12] Phosphonic acids [13-15] are known to form self-assembled monolayers (SAM) on corundum. A similar mechanism is known with alkyl thiols on gold surfaces.[16]

Amino phosphonic acids may be an option to introduce primary amino groups to the surface of the corundum. Subsequently, these groups can be modified with amino-reactive crosslinkers based on N-hydroxysuccinimide esters (NHS esters)[17] or aldehyde groups. Imines, which are the product of the latter with amines, usually need to be stabilized by reduction, preferentially with $NaCNBH_4$, a reaction which is known as reductive amination [18]. In the next step, the surface can be coated with selective binding molecules, such as protein A or G, streptavidin, or antibodies. In a final step, the surface may be blocked to reduce the nonspecific binding (NSB) of irrelevant matrix components. To ensure that the protein of interest can later be eluted exclusively, it is necessary that the (protein) binder is firmly attached to the particles and will not co-elute. In our case, the corundum modified with amino-phosphonic acid was treated with glutaraldehyde, which is a crosslinker widely used in biochemistry for protein bioconjugation. [19,20] Unfortunately, glutaraldehyde is quite toxic and poorly defined chemically as a commercial product. Therefore, a hyperbranched polyglycerol was evaluated on its performance as a less toxic alternative for covalent protein attachment and blocking. Polyglycerols were explored in-depth by the group of Haag et al. and others.[21,22] The relatively high molecular weight and the high number of hydroxyl groups make this group of polymers quite interesting for the derivatization of surfaces in protein chemistry and affinity chromatography.[23] The dendritic structure of these special polyglycerols leads to a beneficial protein-repelling effect when attached to surfaces and therefore suppresses the nonspecific interaction of matrix proteins with the surface.[24,25]

In order to examine the performance of the functionalized corundum surface presented here, the isolation of antibodies from plasma was investigated. A standard method for the binding of IgG is the use of protein A or protein G, coated on different supports. These proteins, originating from the bacteria *Staphylococcus aureus* and *Streptococcus*, selectively bind immunoglobulins (IgG) from different species.[26] Similar isolation of IgG was already performed by using polymer monoliths [27], sintered glass monoliths, both functionalized with protein A[28] and on alumina monoliths with protein G [29]. The present work shows the application of nonporous, functionalized corundum without the demand for a chromatography system and with nearly unlimited potential for scale-up at a very low cost.

2. Materials and Methods

Corundum F1200 was obtained by Hasenfratz Sandstrahltechnik (Aßling, Germany). Hyperbranched polyglycerol (99.4%, 10 kDa) was obtained by DendroPharm GmbH (Berlin, Germany). Other materials and reagents were obtained as follows: Acetonitrile 99.9% (ACN, 2697), hydrochloric acid super pure (34-37% w/w), silver nitrate 99% (7761-88-8)

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were purchased from Th. Geyer GmbH & Co. KG (Renningen, Germany); amino acid standard AAS18, 12-aminododecylphosphonic acid hydrochloride salt 95% (2177270-88-9), bovine serum albumin (BSA) >98% (A7906), ethanol absolute, 99.5 % (64-17-5), glutaraldehyde 50% in water (111-30-8), glycerol for molecular biology ≥99% (56-40-6), DL-methionine ≥99% (59-51-8**),** sodium dodecyl sulfate (151-21-3), Tween 20 (P1379) were purchased from Sigma Aldrich (St. Louis, MO, USA); bromophenol blue (76-59-5) was purchased from Merck KGaA (Darmstadt, Germany), glycine (56-40-6), PBS (1X Dulbecco's)-Powder, tris base (77-86-1) were purchased from AppliChem GmbH (Darmstadt, Germany), human plasma Lyoplas was purchased from DRK-Blutspendedienst West gGmbH, protein A recombinant > 95% PRO-356 was purchased from Prospec-Tany Technogene Ltd. (Ness-Ziona, Israel), Serva Dual Color protein standard III, Quick Coomassie Stain (35081.01) were purchased from Serva Electrophoresis GmbH (Heidelberg, Germany), Pierce BCA Protein Assay Kit (23225), sodium cyanoborohydride 95% (25895-60-7), trifluoroacetic acid 99.5% (TFA, 85183) was obtained from Thermo Fisher (Waltham, MA, USA), lab water was used from a Milli-Q water purification system (Millipore, Bedford, MA, USA) with a resistivity of >18.2 Ω and TOC value of < 5 ppm.

2.1 TEM, ESEM

(Scanning) Transmission electron microscope ((S)TEM) images and scans, as well as energy-dispersive X-ray spectroscopy (EDX) analyses, were obtained with a Talos F200S, 200 kV, (Thermo Fisher Scientific). For the measurements, 1 mg/mL of the corundum suspension was prepared in ethanol, and 10 µL was placed on a copper grid and left for drying. Images were analyzed with ImageJ, version 1.53h (National Institutes of Health and the Laboratory for Optical and Computational Instrumentation) [30].

Environmental Scanning Electron Microscopy (ESEM) characterization was conducted on an XL 30 ESEM equipped with a tungsten cathode (FEI, Eindhoven, electronic upgrade by point electronic GmbH). ESEM investigations were performed in the "high-vacuum mode" of the microscope. The signal was recorded with a secondary electron detector (SE). Before the analysis, all samples were coated with a 15 nm thick layer of gold.

2.2 Dynamic Light Scattering and zeta potential measurements

Dynamic light scattering experiments were performed at an Anton Paar Lite-Sizer 500 instrument with disposable cuvettes made from polystyrene (10 mm) purchased from Th. Geyer GmbH & Co. KG (Renningen, Germany). A 1% w/w corundum was prepared in PBS pH 7.4, which was filtered through a 2 µm filter in advance, and then diluted in a ratio of 1:500 in filtered PBS and tempered to 25 °C. Measurements were performed in a forward scatter mode and the advanced cumulant model. The zeta potential measurements were performed at a Zetasizer Malvern Instrument with a Malvern Panalytical-Disposable Capillary Cell (DTS1061). The target temperature was 25 °C, and the Smoluchowski approximation was used. The 0.1% w/w corundum solutions with different functionalizations, suspended in 1 mM KCl pH 7, which was filtered through a 2 µm filter, were diluted in a ratio of 1:50 in filtered 1 mM KCl.

2.3 BET

BET (Brunauer-Emmett-Teller Theory**)** measurements were performed on the ASAP2020, Fa. Micromeritics (Norcross, USA) with N_2 and an analysis bath temperature of 77.3 K. Corundum particles were dried at 250 °C for 17 h and baked out in a vacuum. The BET-range was $0.07 \leq p/p_0 < 0.20$**.** The linear plot of the isotherm and the BET surface area plot are shown in the supplementary materials.

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2.4 Purification protocol for raw corundum

In order to eliminate residual impurities of the corundum particles, 1 g of the material was incubated with 5 mL 10% KOH solution for 10 min at RT. After centrifugation and removal of the supernatant, the particles were dispersed in 5 mL 37% hydrochloric acid and transferred to hydrolysis glass tubes. In the hydrolysis setup, which is made of a custom-made copper block, the sample was heated to 100 °C for 20 min. After dilution in 1:1 lab water, the dispersions were transferred into centrifuge tubes and washed thoroughly with 5 mL lab water three times before they could be used for further functionalization.

2.5 Functionalization of corundum with 12-aminododecylphosphonic acid hydrochloride (12-APA) and glutaraldehyde/oxidized polyglycerol for the conjugation with BSA/ protein A

1 g of purified corundum was incubated with 2 mL of a 36 mM 12-APA-solution in ethanol. The dispersion was kept at RT in an overhead shaker for 16 hours (20 rpm). An oxidation step has to be performed to partially oxidize the diols of polyglycerol into aldehyde groups for further conjugation. To a 1% polyglycerol solution (100 mg in 10 mL PBS pH 7.4), 106 mg of sodium periodate was added and kept at RT in an overhead shaker for 16 hours (20 rpm). For 100 mg of PG, which contains 1.35 mmol hydroxyl groups, 0.5 mmol of sodium periodate was used. The corundum particles were then washed three times with ethanol and once with lab water. In the following step, the 12-APA modified particles were incubated with either 10 mL of a solution containing 1% of oxidized polyglycerol or with a 1% glutaraldehyde solution in PBS. The incubation took place for 3 hours at RT, followed by three washing steps with PBS. The particles were then incubated with either 5 mL of a 2 mg/mL BSA in PBS solution or 3 mL of a 3 mg/mL protein A in PBS solution. Protein conjugation was performed over 16 hours at RT. Without further washing steps, the particles were incubated with 10 mL of a 50 mM $NaCNBH_3$ solution and kept in an overhead shaker for 1 hour. After three washing steps with PBST (0.1% Tween 20) and a wash with lab water, the functionalized particles were ready for further experiments.

For the IgG affinity enrichment experiment, human plasma was diluted 1:10 in PBS, and 10 mL (containing approximately 10 mg of IgG) were incubated with 1 g of protein-A-functionalized corundum particles. After incubation for 1 hour, the particles were washed three times with PBST (0.1% Tween 20) and one time with lab water.

2.6 Aromatic amino acid analysis and HPLC analysis

Aromatic amino acid analysis (AAAA) was performed as previously described.[31,32] A dispersion of 1 mL of protein-functionalized corundum containing 1 g corundum particles was transferred into hydrolysis glass tubes, and 6 mL of 37% hydrochloric acid containing 17 mg/mL of DL-methionine were added. The air in the glass tubes was displaced by an argon stream applied for 60 seconds. The glass tubes were placed into a copper block and heated under stirring for 1 hour at 150°C. After cooling down, the hydrolysis solution was diluted at 1:5 in lab water and centrifuged at 18,000 rpm for 15 min. Diluted samples were filled into HPLC glass vials for quantification.

For HPLC analysis, an Agilent 1260 (Agilent Technologies, Santa Clara, USA) with the software Agilent Open LAB CDS (Version A.04.06) was used. The column used for separation was purchased from Agilent (AdvanceBio Peptide Mapping Column, 2.1 x 150 mm, 2,7 µm). The detection of the tyrosine peak was performed with the FLD system at an excitation wavelength of 272 nm and an emission wavelength of 303 nm. The separation was achieved under isocratic conditions for 10 min with a mixture of the mobile phase of 99% lab water with 0.1% TFA and 1% ACN with 0.85% TFA. A washing step with 90% ACN/10% lab water was followed to clean the column. Calibration took place with the

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amino acid standard listed above. Calibration curves can be found in the supplementary materials.

2.7 IgG affinity enrichment SDS-PAGE

SDS-PAGE was performed with the electrophoresis system XCell SureLock from Invitrogen AG (Waltham, USA) with the gel Novex WedgeWell 8 to 16%, tris-glycine, 1.0 mm, Mini Protein Gel, 10-well, XP08160BOX. Functionalized particles were incubated with 1.25 mL of SDS loading buffer (4x concentrated: tris base (252 mM, pH 6.8), glycerol (40%), SDS (8%), bromophenol blue (0.02%), in lab water) and 3.75 mL PBS and kept in an overhead shaker for 15 min at RT. After centrifugation (13000 rpm, 5 min), the eluates and all of the reference samples were heated to 90 °C for 5 min before they were loaded onto the gel. For the staining, the Quick Coomassie Stain (35081.01 von Serva) was used before the gel was stained with silver. The staining solutions (150 mL each) were prepared as follows: (1) 0.02% sodium thiosulfate, (2) 0.1% silver nitrate, (3) 2.5% sodium carbonate, (4) 2.5% sodium carbonate and 0.02% formaldehyde, (5) 50mM EDTA disodium salt. The Coomassie-stained gel was incubated with solution (1) for 1 minute and washed two times with lab water. The incubation with solution (2) took place for 25 min before washing once with lab water. It followed incubation with solution (3) for 10 seconds before solution (4) was added. The gel was stained for 10 min before another washing step, and the incubation with solution (5) took place for 5 min. The gel was then finally washed with lab water.

For the determination of the IgG-binding capacity, the corundum was treated with 5 mL glycine/HCl elution buffer pH 2.3 for 15 min at RT. The eluate was neutralized with 20 µL 5 M NaOH to pH 7, and the corundum was removed by centrifugation (13000 rpm, 5 min). The IgG concentration of the eluate was determined with a BCA assay according to the protocol from Pierce BCA Protein Assay Kit (MAN0011430). For the test, 25 µL per sample and BSA standard were pipetted into a microplate well before 200 µL of working reagent containing BCA reagents A and B (50:1) was added. The microplate was then incubated for 30 min at 37 °C. The absorbance was measured at 562 nm at the microplate UV-Vis reader BioTek Epoch 2 with the software Gen 5 (Version 2.07). BSA calibration values were multiplied with the factor of 1.09 as the absorbance ratio of human IgG relative to BSA.

3. Results

3.1 Particles characterization of corundum

The corundum raw material used in this work is an industrial product for abrasive purposes. TEM, ESEM, BET, DLS, and zeta potential measurements were performed to characterize the corundum particles and give insight into the shape, size, and surface properties of this material. Before functionalization, the particles were purified, as shown in the supplementary material. In a BET measurement, a surface area of 4.5 m^2/g was determined**.** DLS measurements showed an average particle size of 0.78 µm ± 0.14 (supplementary materials). However, TEM and ESEM micrographs revealed that a broad range of particle sizes and shapes are present due to the milling process. Figure 1 shows the macroscopic view of the raw material (a), a TEM micrograph for a corundum particle (b) as well as an ESEM micrograph (c). The surface of the particle appears to be nonporous, and the particles have sharp edges. These images are consistent with the mean particle sizes and surface area determined by DLS and BET.

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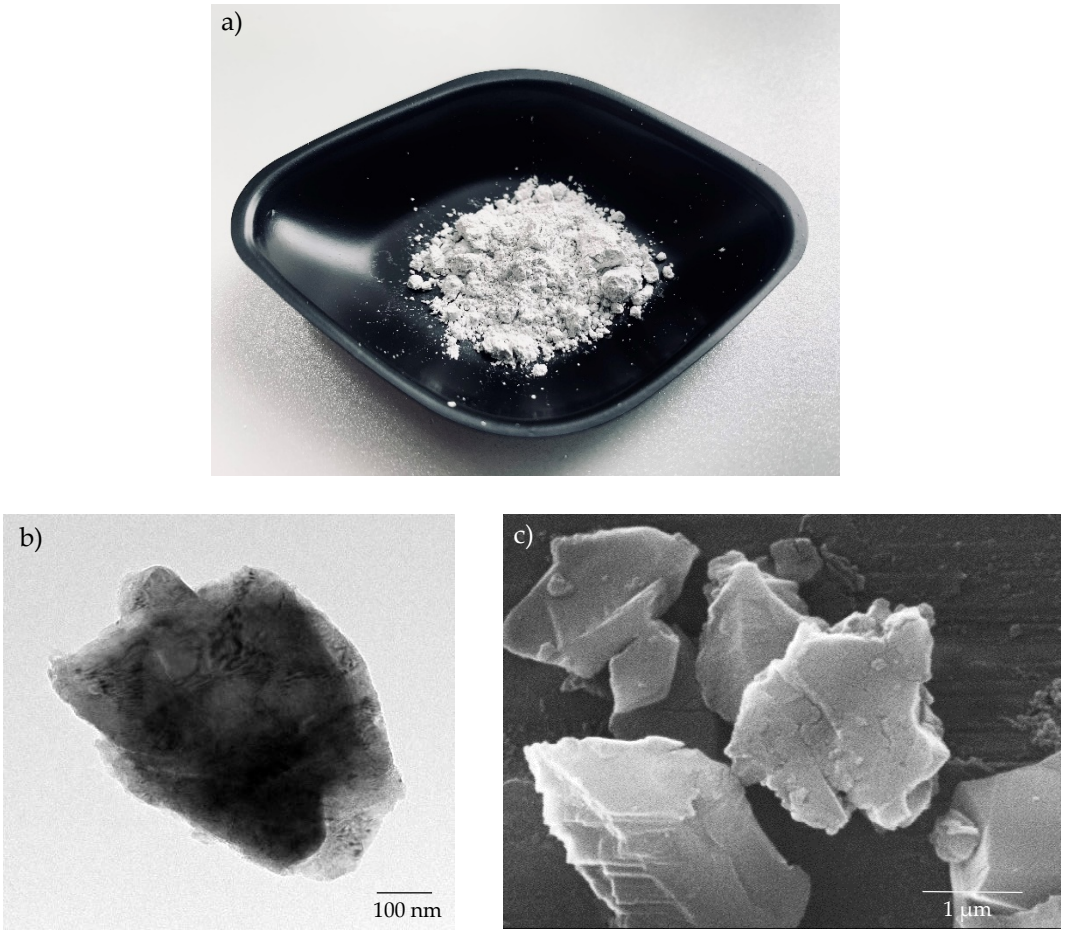

Figure 1. Macroscopic view of the raw corundum powder (**a**) TEM micrograph and (**b**) ESEM micrograph (**c**) showing purified particles with an irregular shape but a smooth, nonporous surface.

3.2 Surface functionalization with amino phosphonic acids/glutaraldehyde and oxidized hyperbranched polyglycerol

In order to immobilize proteins or peptides on the corundum surface covalently, 12-aminododecylphosphonic acid (12-APA) was used as an interface to provide a firmly bound functional group that can be modified in further steps. Once the phosphonic acid is attached to the surface, a further reaction with a crosslinker is necessary to link the desired protein to the corundum surface. This was achieved by using the well-established bifunctional crosslinker glutaraldehyde, which will react in a nucleophilic addition reaction with the amine group of the phosphonic acid and in the same manner with the amino groups of protein mainly provided by lysine residues. A hyperbranched polyglycerol (PG) was explored in this work to find an alternative to the toxic and poorly defined glutaraldehyde. After some diols are cleaved to aldehyde groups, the PG is allowed to react in a similar manner as the glutaraldehyde does, with the exception that the PG is highly branched in structure. Aside from the expected lower toxicity, it was expected to lead to a higher protein binding capacity due to the existence of more functional groups that can be linked to the protein. The PG was oxidized for 16 hours with a molar ratio of 1.4:1 (hydroxyl groups/periodate) in PBS. Our results suggest that oxidation overnight led to higher protein binding capacities than oxidation for one hour (data not shown). Figure 2 shows the oxidation reaction of the diol cleavage by $NaIO_4$. In this reaction, the diols are cleaved, resulting in aldehyde groups that can be modified later on.

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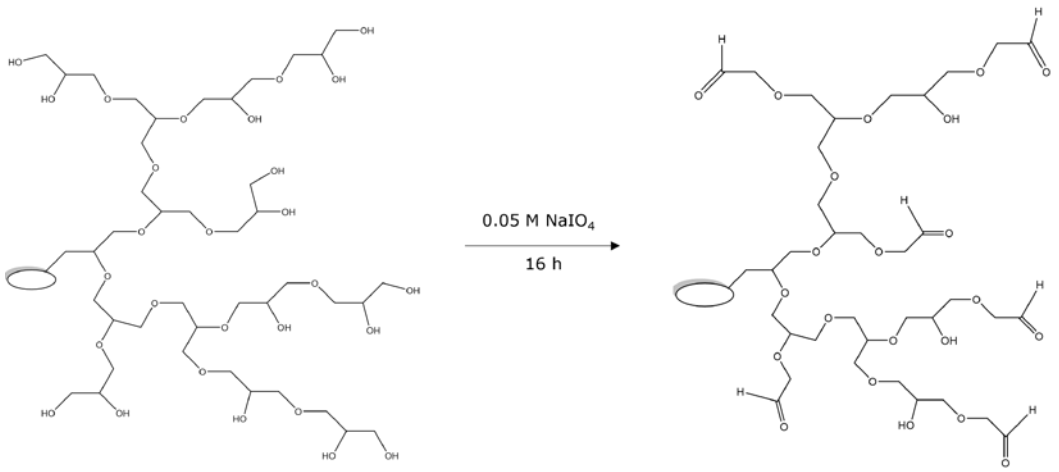

Figure 2. Oxidation of diols in polyglycerol with sodium periodate.

The oxidized PG was then incubated in the same way as glutaraldehyde with the amino phosphonic acid-functionalized corundum. Zeta potential measurements were performed to characterize the change in the surface chemistry (Table 1).

Table 1. Zeta potential measurements of unfunctionalized and functionalized corundum. Measurements were performed in 1 mM KCl at a pH of 7.

sample	zeta potential [mV]
Corundum	4.28 ± 0.01
Corundum - 12-APA	-2.94 ± 0.37
Corundum - 12-APA - glutaraldehyde	-15.00 ± 0.52
Corundum - 12-APA - oxidized polyglycerol	-15.10 ± 3.70

These values clearly show a drop in the surface charge from a slightly positive zeta potential of plain corundum to negative values after coating with 12-APA, glutaraldehyde, and polyglycerol. A reduction of the imines by $NaCNBH_3$ was not performed for these samples.

3.2 Protein immobilization and direct quantification with aromatic amino acid analysis (AAAA)

Bovine serum albumin (BSA) was used as a model protein to determine which of the tested conjugation strategy offers the highest protein binding capacity. Figure 3 shows the functionalization schemes for corundum.

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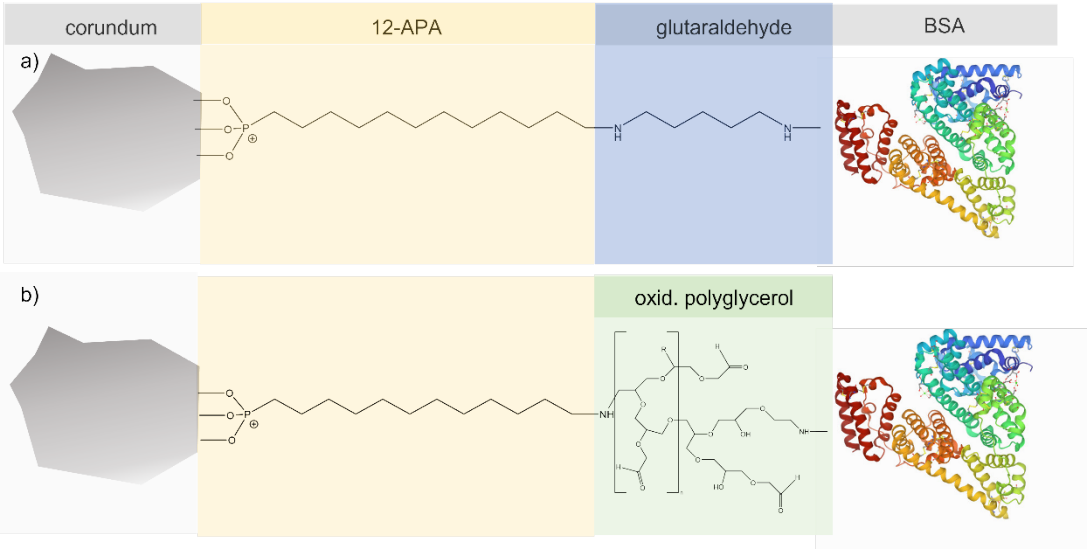

Figure 3. Scheme of corundum particles modified with 12-aminododecylphosphonic acid (12-APA), the (**a**) crosslinkers glutaraldehyde, or (**b**) oxidized polyglycerol, respectively, finally conjugated to bovine serum albumin (BSA); Structures are not to scale; BSA structure from [33].

Direct protein quantification on particles is an elegant way to circumvent the challenges that are faced when trying to quantify the unbound amount of protein in the supernatant. In a recent work by Tchipilov et al., the disadvantages of such an indirect approach to quantification were discussed in detail.[34] Aromatic amino acid analysis (AAAA), in contrast, is a useful and very precise approach for direct protein quantification in liquids or on solid surfaces. The particles coated with proteins were dispersed in 37% hydrochloric acid containing DL-methionine as a reductant to protect sensitive amino acids from oxidative degradation. The mixture was heated in a copper block for 1 hour at 150 °C. After centrifugation to remove the corundum particles, the hydrolyzed protein solution is diluted and injected into an HPLC system for quantification. The amino acid sequence of the protein BSA is known, and therefore the number of the respective aromatic amino acids, too. The total protein content of the sample can be calculated with the use of an external aromatic amino acid calibration. In this work, the fluorescence detection of tyrosine was used to calculate the amount of immobilized protein on the corundum particles. Figure 4 illustrates the described workflow.

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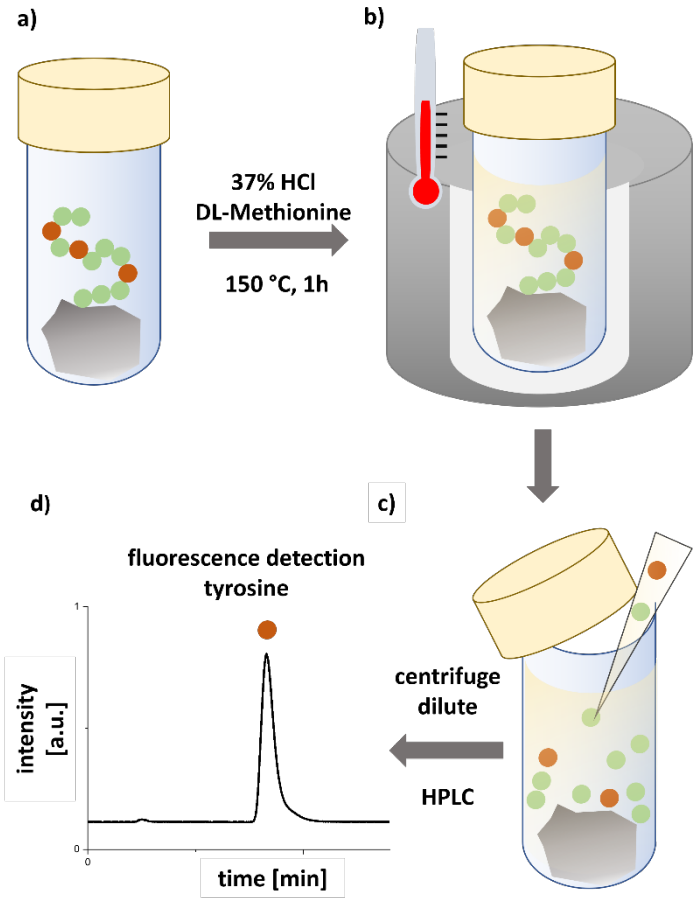

Figure 4. Workflow of aromatic amino acid analysis (AAAA) of protein-functionalized corundum. Red dots symbolize aromatic amino acids in the sequence. (**a**) protein bound to corundum particles (**b**) hydrolysis in HCl at 150°C for 1 h (**c**) dilution of hydrolysis supernatant (**d**) HPLC analysis of tyrosine with fluorescence detection (excitation 272 nm/ emission 303 nm).

The amount of immobilized BSA was calculated with a calibration based on a standard amino acid solution containing L-tyrosine. Quantification of BSA on 12-APA-modified corundum showed a value of 2.54 mg ± 0.02 when functionalized with oxidized polyglycerol and 1.96 mg ± 0.02 with glutaraldehyde. This data suggest that the use of 12-APA in combination with oxidized polyglycerol can bind more BSA than glutaraldehyde.

3.3 Affinity enrichment of IgG from human plasma with protein-A-functionalized corundum

For a proof of concept experiment, IgG should be isolated from human plasma with 12-APA-modified corundum particles that were functionalized with oxidized polyglycerol (PG) and subsequently conjugated to protein A. Protein A selectively binds the F_c part of immunoglobulins. Therefore it can be used as an affinity binder to isolate IgG from a complex matrix such as plasma which is estimated to contain up to 10,000 different proteins [35]. The amount of immobilized protein A was quantified with the previously described method. An amount of 0.5 ± 0.04 mg protein A per 1 g corundum was determined. Human plasma was diluted at 1:10 and incubated with the functionalized particles. After several washing steps and centrifugation, the supernatant containing the remaining proteins of the human plasma was discarded. The particles were then treated with a sodium dodecyl sulfate (SDS) solution (2% tris base pH 6.8) and incubated for 15 min to elute the bound IgG. The solution was separated from the corundum via

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centrifugation and then heated for complete protein denaturation before it was loaded onto the SDS gel. Figure 5 shows the described workflow and the SDS gel obtained in this experiment.

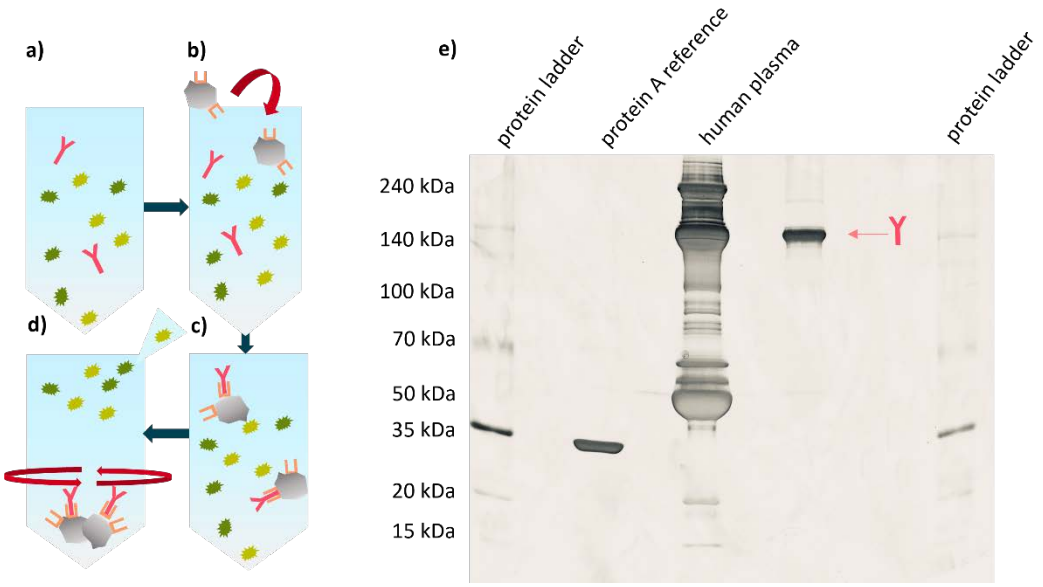

Figure 5. Affinity enrichment of IgG (Y symbol) from human plasma. Corundum was coated with 12-APA and oxidized polyglycerol. **a)** human plasma containing a high number of different proteins **b)** incubation with protein-A-functionalized corundum. **c)** binding of IgG to protein A (U symbol) **d)** centrifugation and removal of supernatant **e)** SDS-PAGE after the elution of bound IgG by a 2% SDS solution.

The silver-stained SDS-PAGE separation indicates the successful isolation of IgG from human plasma. In addition, no protein A leakage was observed, confirming the covalent attachment of protein A to the oxidized polyglycerol.

To determine the IgG-binding capacity, protein A functionalized corundum which was incubated with 1:10 diluted human plasma for 1 h, was washed three times with PBST and once with lab water before it was treated with glycine/HCl buffer of pH 2.3 for 15 min. After neutralization, the amount of 1.80 mg ± 0.01 IgG per 1 g corundum was determined with a BCA commercial assay.

4. Discussion

In this work, corundum powder, intended to be used in industrial applications, was purified, characterized and functionalized to create a new platform for the isolation and enrichment of targeted proteins from complex matrixes like human plasma. This approach leads to an affinity method that can be easily performed in any lab possessing a standard centrifuge. This makes protein isolation independent of access to complex column-based instruments or magnetic separation systems.

The raw corundum material showed some impurities in the TEM micrographs. Therefore, a brief purification protocol was established. The functionalization with a long-chain amino phosphonic acid was used to generate a surface with primary amine groups. In a subsequent step, glutaraldehyde was compared with oxidized polyglycerol (PG) as linkers. Our results showed that in direct comparison, the oxidized PG could bind more BSA than glutaraldehyde, making it a viable alternative. In a next experiment, protein A was conjugated to corundum modified with 12-APA and oxidized PG to isolate IgG from hu-

man plasma. The results showed the successful affinity enrichment of IgG with high purity. No protein A leakage was detected even under harsh conditions, which confirms the covalent attachment of protein A to the corundum particles. Furthermore, the nonspecific binding (NSB) of matrix proteins was found to be very low, which shows that the oxidized PG also acts as an excellent surface coating. These proof-of-concept experiments demonstrate the performance and flexibility of these novel materials based on corundum as a platform for affinity separations.

Supplementary Materials: The following supporting information can be downloaded at: www.mdpi.com/xxx/s1, Figure S1. Dynamic light scattering (DLS) measurements of corundum F1200 in 0.1 M PBS indicated an average particle size of 1.2 µm. Figure S2. TEM images of unpurified and purified corundum, Corundum surface covered with needle-shaped crystals a). Elemental mapping showing sodium- and chloride-containing structures b). Figure S3. Purification protocol for corundum. Figure S4. BET measurements. Isotherm linear plot and BET surface area plot. Figure S5**.** Calibration line of tyrosine (amino acid standard solution). Fluorescence detection was performed at 272 nm (Ex.) and 303 nm (Em.).

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