Assessing the biocompatibility and stability of CeO_2 nanoparticle conjugates with aza-crowns for use as a radiopharmaceutical

Supplementary Information

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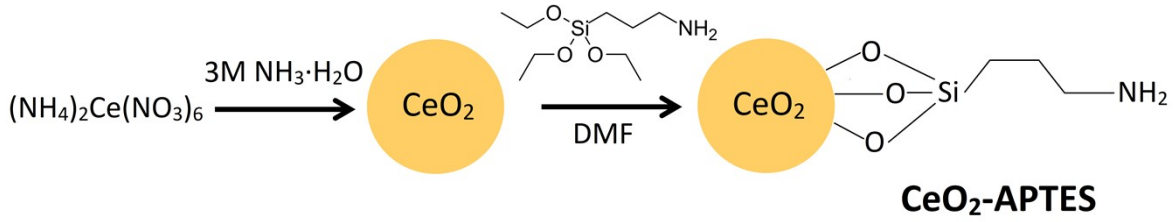
Surface modification CeO_2 by the linker APTES

Various methods of modification of the cerium oxide surface by the APTES were used, differing in the ratio of the starting substances (Table S1). CeO_2 nanoparticles were suspended in anhydrous DMF and then the required amount of APTES was added and stirred at 40°C for 12 h. The resulting materials were separated, washed four times with DMF and once with acetone to remove excess DMF and dried. The scheme of functionalisation of the surface of CeO_2 nanoparticles by linker is shown in Scheme S1.

Table S1. Reagent ratios in the synthesis of modified CeO_2-APTES nanoparticles.

The resulting materials	m(CeO2), mg	V(DMF), *μL*	V(APTES), *μL*	V(APTES)/ V(DMF), %	Synthesis temperature
CeO_2-APTES_1	10	1000	30	3	40 °C
CeO_2-APTES_2	10	1000	3	0,3	40 °C



Scheme S1. Schematic showing the modification of CeO_2 nanoparticles with the APTES linker.

The degree of amine grafting on the surface of cerium dioxide nanoparticles available for ligands conjugation was determined by using the ninhydrin test based on the reaction of primary amino groups with ninhydrin and the formation of a coloured purple compound. Initially, a calibration curve was plotted (Fig. S1 A). 0.2% ninhydrin solution was added to a series of glycine solutions in 50% ethanol in a volume ratio of 1:1 (Table S2). The resulting solutions were mixed at 90°C

for 5 min. The absorption spectra of solutions in the 350-700 nm region were recorded after cooling to room temperature. The maximum absorption of the ninhydrin reaction product is observed at a wavelength of 570 nm.

Table S2. Calibration curve parameters for glycine solutions.

$C_{glycine}$, μg/ml	3.0	5.0	10.0	100.0
$C_{amino\ group}$, μg/ ml	0.53	1.07	2.13	21.33
Absorbance, a.u.	0	0.01	0.02	0.23

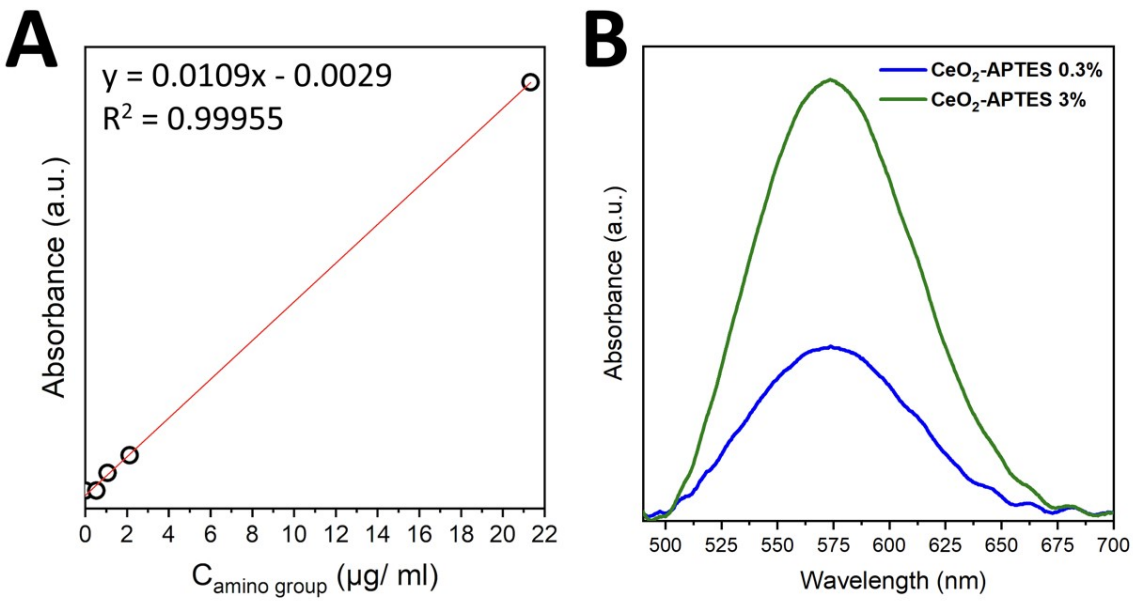


Fig. S1. (A) Dependence absorbance of the concentration of amino groups in solution. (B) Absorbance curves of the ninhydrin reaction products of CeO_2-APTES in the ratios of 3 and 0.3% (V_{APTES}/V_{DMF}).

The coefficient k = $1.09 \cdot 10^{-2}$ ml/(μg*cm) was defined, which allows determining the number of amino groups on the surface of nanoparticles by determining the dependence of absorbance from the concentration of amino groups in solution.

The modified nanoparticles CeO_2-APTES_1 and CeO_2-APTES_2 were dispersed with the same concentrations to determine the number of amino groups on the surface of CeO_2. Then, an equal volume of 0.2% ninhydrin solution was added to the suspension of nanoparticles, and the mixtures were kept at 90°C for 5 minutes and cooled to room temperature. Nanoparticles were separated by centrifugation to obtain correct data. The concentrations of amino groups in the volume of the supernatant and of APTES per unit mass of nanoparticles (mg/g) were determined by the spectrophotometer (Fig. S1 B).

The maximum absorption can be detected in the samples obtained at a ratio of 3% and 0.3%. According to the spectrophotometer data, there are more amino groups in the sample obtained in a volume ratio of 3%. The concentration of amino groups on the surface CeO_2 is 0.82 μg/ml and 1.71 μg/ml, respectively.

The volume APTES/DMF ratio of 3% was selected for further experiments.

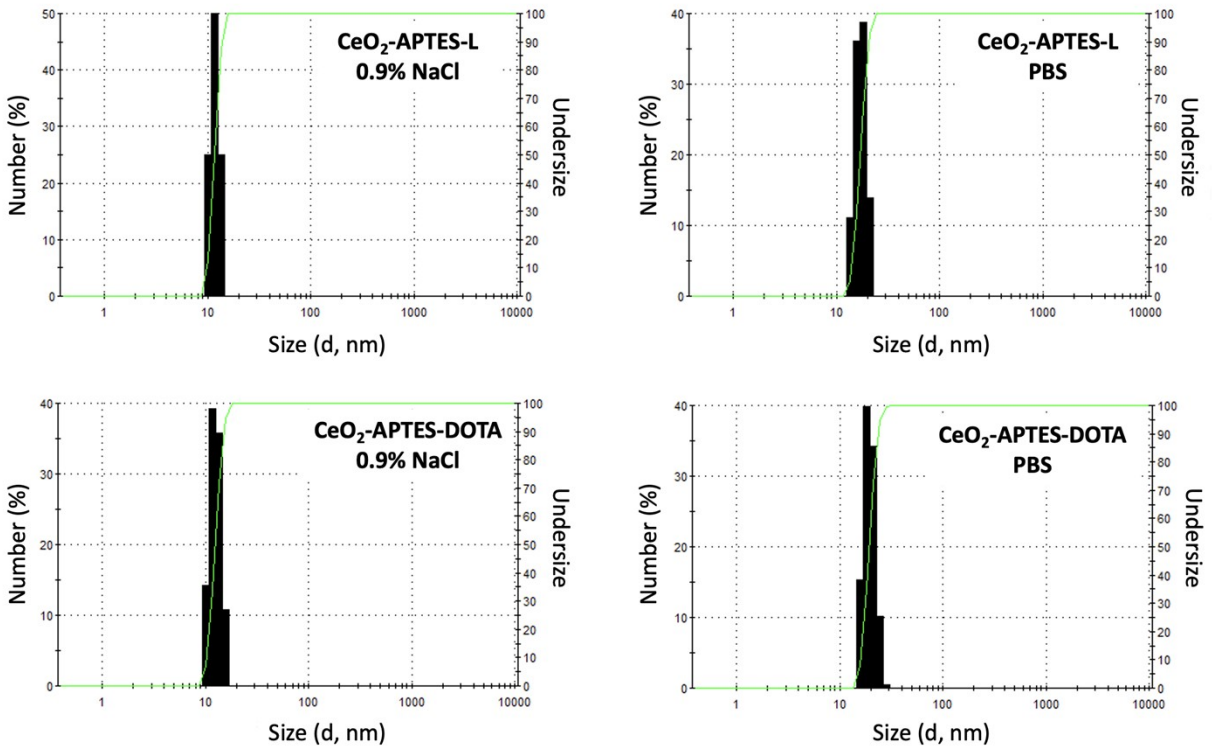


Fig. S2. CeO_2-APTES-L and CeO_2-APTES-DOTA size distribution in the different solutions according to DLS data.